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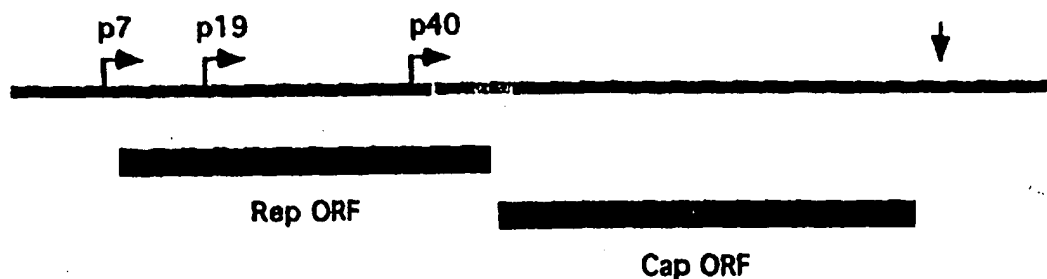


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<p>(21) International Application Number: PCT/US97/16266 (22) International Filing Date: 11 September 1997 (11.09.97) (30) Priority Data: 60/025,934 11 September 1996 (11.09.96) US (71) Applicant (for all designated States except US): THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; National Institutes of Health, Office of Technology Transfer, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): CHIORINI, John, A. [US/US]; 2604 Loma Street, Silver Spring, MD 20902 (US). KOTIN, Robert, M. [US/US]; 707 Gormley, Rockville, MD 20850 (US). SAFER, Brian [US/US]; 1610 Tilton Drive, Silver Springs, MD 20902 (US). (74) Agents: SELBY, Elizabeth et al.; Needle & Rosenberg, Suite 1200, 127 Peachtree Street, N.E., Atlanta, GA 30303 (US).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published Without international search report and to be republished upon receipt of that report.</p>

(54) Title: AAV4 VECTOR AND USES THEREOF



(57) Abstract

The present invention provides an adeno-associated virus 4 (AAV4) virus and vectors and particles derived therefrom. In addition, the present invention provides methods of delivering a nucleic acid to a cell using the AAV4 vectors and particles.

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AAV4 VECTOR AND USES THEREOF

BACKGROUND OF THE INVENTION

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Field of the Invention

The present invention provides adeno-associated virus 4 (AAV4) and vectors derived therefrom. Thus, the present invention relates to AAV4 vectors for and
10 methods of delivering nucleic acids to cells of subjects.

Background Art

Adeno associated virus (AAV) is a small nonpathogenic virus of the parvoviridae
15 family (for review see 28). AAV is distinct from the other members of this family by its dependence upon a helper virus for replication. In the absence of a helper virus, AAV may integrate in a locus specific manner into the q arm of chromosome 19 (21). The approximately 5 kb genome of AAV consists of one segment of single stranded DNA of either plus or minus polarity. The ends of the genome are short inverted terminal
20 repeats which can fold into hairpin structures and serve as the origin of viral DNA replication. Physically, the parvovirus virion is non-enveloped and its icosohedral capsid is approximately 20 nm in diameter.

To date 7 serologically distinct AAVs have been identified and 5 have been
25 isolated from humans or primates and are referred to as AAV types 1-5 (1). The most extensively studied of these isolates is AAV type 2 (AAV2). The genome of AAV2 is 4680 nucleotides in length and contains two open reading frames (ORFs). The left ORF encodes the non-structural Rep proteins, Rep40, Rep 52, Rep68 and Rep 78, which are involved in regulation of replication and transcription in addition to the production of
30 single-stranded progeny genomes (5-8, 11, 12, 15, 17, 19, 21-23, 25, 34, 37-40). Furthermore, two of the Rep proteins have been associated with the preferential

integration of AAV genomes into a region of the q arm of human chromosome 19. Rep68/78 have also been shown to possess NTP binding activity as well as DNA and RNA helicase activities. The Rep proteins possess a nuclear localization signal as well as several potential phosphorylation sites. Mutation of one of these kinase sites resulted in a loss of replication activity.

The ends of the genome are short inverted terminal repeats which have the potential to fold into T-shaped hairpin structures that serve as the origin of viral DNA replication. Within the ITR region two elements have been described which are central to the function of the ITR, a GAGC repeat motif and the terminal resolution site (trs). The repeat motif has been shown to bind Rep when the ITR is in either a linear or hairpin conformation (7, 8, 26). This binding serves to position Rep68/78 for cleavage at the trs which occurs in a site- and strand-specific manner. In addition to their role in replication, these two elements appear to be central to viral integration. Contained within the chromosome 19 integration locus is a Rep binding site with an adjacent trs. These elements have been shown to be functional and necessary for locus specific integration.

The AAV2 virion is a non-enveloped, icosohedral particle approximately 25 nm in diameter, consisting of three related proteins referred to as VP1, 2 and 3. The right ORF encodes the capsid proteins, VP1, VP2, and VP3. These proteins are found in a ratio of 1:1:10 respectively and are all derived from the right-hand ORF. The capsid proteins differ from each other by the use of alternative splicing and an unusual start codon. Deletion analysis has shown that removal or alteration of VP1 which is translated from an alternatively spliced message results in a reduced yield of infectious particles (15, 16, 38). Mutations within the VP3 coding region result in the failure to produce any single-stranded progeny DNA or infectious particles (15, 16, 38).

The following features of AAV have made it an attractive vector for gene transfer (16). AAV vectors have been shown *in vitro* to stably integrate into the cellular genome; possess a broad host range; transduce both dividing and non dividing cells *in vitro* and *in vivo* (13, 20, 30, 32) and maintain high levels of expression of the transduced genes (41). Viral particles are heat stable, resistant to solvents, detergents, changes in pH, temperature, and can be concentrated on CsCl gradients (1,2).

Integration of AAV provirus is not associated with any long term negative effects on cell growth or differentiation (3,42). The ITRs have been shown to be the only *cis* elements required for replication, packaging and integration (35) and may contain some promoter activities (14).

5

Initial data indicate that AAV4 is a unique member of this family. DNA hybridization data indicated a similar level of homology for AAV1-4 (31). However, in contrast to the other AAVs only one ORF corresponding to the capsid proteins was identified in AAV4 and no ORF was detected for the Rep proteins (27).

10

AAV2 was originally thought to infect a wide variety of cell types provided the appropriate helper virus was present. Recent work has shown that some cell lines are transduced very poorly by AAV2 (30). While the receptor has not been completely characterized, binding studies have indicated that it is poorly expressed on erythroid cells (26). Recombinant AAV2 transduction of CD34⁺, bone marrow pluripotent cells, requires a multiplicity of infection (MOI) of 10⁴ particles per cell (A. W. Nienhuis unpublished results). This suggests that transduction is occurring by a non-specific mechanism or that the density of receptors displayed on the cell surface is low compared to other cell types.

20

The present invention provides a vector comprising the AAV4 virus as well as AAV4 viral particles. While AAV4 is similar to AAV2, the two viruses are found herein to be physically and genetically distinct. These differences endow AAV4 with some unique advantages which better suit it as a vector for gene therapy. For example, the wt AAV4 genome is larger than AAV2, allowing for efficient encapsidation of a larger recombinant genome. Furthermore, wt AAV4 particles have a greater buoyant density than AAV2 particles and therefore are more easily separated from contaminating helper virus and empty AAV particles than AAV2-based particles. Additionally, in contrast to AAV1, 2, and 3, AAV4, is able to hemagglutinate human, guinea pig, and sheep erythrocytes (18).

30

Furthermore, as shown herein, AAV4 capsid protein, again surprisingly, is distinct from AAV2 capsid protein and exhibits different tissue tropism. AAV2 and AAV4 have been shown to be serologically distinct and thus, in a gene therapy application, AAV4 would allow for transduction of a patient who already possess
5 neutralizing antibodies to AAV2 either as a result of natural immunological defense or from prior exposure to AAV2 vectors. Thus, the present invention, by providing these new recombinant vectors and particles based on AAV4 provides a new and highly useful series of vectors.

SUMMARY OF THE INVENTION

The present invention provides a nucleic acid vector comprising a pair of adeno-
5 associated virus 4 (AAV4) inverted terminal repeats and a promoter between the
inverted terminal repeats.

The present invention further provides an AAV4 particle containing a vector
comprising a pair of AAV2 inverted terminal repeats.

10

Additionally, the instant invention provides an isolated nucleic acid comprising
the nucleotide sequence set forth in SEQ ID NO:1 [AAV4 genome]. Furthermore, the
present invention provides an isolated nucleic acid consisting essentially of the
nucleotide sequence set forth in SEQ ID NO:1 [AAV4 genome].

15

The present invention provides an isolated nucleic acid encoding an adeno-
associated virus 4 Rep protein. Additionally provided is an isolated AAV4 Rep protein
having the amino acid sequence set forth in SEQ ID NO:2, or a unique fragment thereof.
Additionally provided is an isolated AAV4 Rep protein having the amino acid sequence
20 set forth in SEQ ID NO:8, or a unique fragment thereof. Additionally provided is an
isolated AAV4 Rep protein having the amino acid sequence set forth in SEQ ID NO:9,
or a unique fragment thereof. Additionally provided is an isolated AAV4 Rep protein
having the amino acid sequence set forth in SEQ ID NO:10, or a unique fragment
thereof. Additionally provided is an isolated AAV4 Rep protein having the amino acid
25 sequence set forth in SEQ ID NO:11, or a unique fragment thereof.

The present invention further provides an isolated AAV4 capsid protein having
the amino acid sequence set forth in SEQ ID NO:4. Additionally provided is an isolated
AAV4 capsid protein having the amino acid sequence set forth in SEQ ID NO:16. Also
30 provided is an isolated AAV4 capsid protein having the amino acid sequence set forth in
SEQ ID NO:18.

The present invention additionally provides an isolated nucleic acid encoding adeno-associated virus 4 capsid protein.

The present invention further provides an AAV4 particle comprising a capsid
5 protein consisting essentially of the amino acid sequence set forth in SEQ ID NO:4.

Additionally provided by the present invention is an isolated nucleic acid comprising an AAV4 p5 promoter.

10 The instant invention provides a method of screening a cell for infectivity by AAV4 comprising contacting the cell with AAV4 and detecting the presence of AAV4 in the cells.

The present invention further provides a method of delivering a nucleic acid to a
15 cell comprising administering to the cell an AAV4 particle containing a vector comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, thereby delivering the nucleic acid to the cell.

The present invention also provides a method of delivering a nucleic acid to a
20 subject comprising administering to a cell from the subject an AAV4 particle comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, and returning the cell to the subject, thereby delivering the nucleic acid to the subject.

The present invention further provides a method of delivering a nucleic acid to a
25 subject comprising administering to a cell from the subject an AAV4 particle comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, and returning the cell to the subject, thereby delivering the nucleic acid to the subject.

The present invention also provides a method of delivering a nucleic acid to a
30 cell in a subject comprising administering to the subject an AAV4 particle comprising

the nucleic acid inserted between a pair of AAV inverted terminal repeats, thereby delivering the nucleic acid to a cell in the subject.

The instant invention further provides a method of delivering a nucleic acid to a
5 cell in a subject having antibodies to AAV2 comprising administering to the subject an
AAV4 particle comprising the nucleic acid, thereby delivering the nucleic acid to a cell
in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a schematic outline of AAV 4. Promoters are indicated by horizontal arrows with their corresponding map positions indicated above. The polyadenylation site is indicated by a vertical arrow and the two open reading frames are indicated by black boxes. The splice region is indicated by a shaded box.

Fig. 2 shows AAV4 ITR. The sequence of the ITR (SEQ ID NO: 20) is shown in the hairpin conformation. The putative Rep binding site is boxed. The cleavage site in the trs is indicated by an arrow. Bases which differ from the ITR of AAV2 are outlined.

Fig. 3 shows cotransduction of rAAV2 and rAAV4. Cos cells were transduced with a constant amount of rAAV2 or rAAV4 expressing beta galactosidase and increasing amounts of rAAV2 expressing human factor IX (rAAV2FIX). For the competition the number of positive cells detected in the cotransduced wells was divided by the number of positive cells in the control wells (cells transduced with only rAAV2LacZ or rAAV4LacZ) and expressed as a percent of the control. This value was plotted against the number of particles of rAAV2FIX.

Fig. 4 shows effect of trypsin treatment on cos cell transduction. Cos cell monolayers were trypsinized and diluted in complete media. Cells were incubated with virus at an MOI of 260 and following cell attachment the virus was removed. As a control an equal number of cos cells were plated and allowed to attach overnight before transduction with virus for the same amount of time. The number of positive cells was determined by staining 50 hrs post transduction. The data is presented as a ratio of the number of positive cells seen with the trypsinized group and the control group.

DETAILED DESCRIPTION OF THE INVENTION

As used in the specification and in the claims, "a" can mean one or more,
5 depending upon the context in which it is used.

The present invention provides the nucleotide sequence of the adeno-associated virus 4 (AAV4) genome and vectors and particles derived therefrom. Specifically, the present invention provides a nucleic acid vector comprising a pair of AAV4 inverted
10 terminal repeats (ITRs) and a promoter between the inverted terminal repeats. The AAV4 ITRs are exemplified by the nucleotide sequence set forth in SEQ ID NO:6 and SEQ ID NO:20; however, these sequences can have minor modifications and still be contemplated to constitute AAV4 ITRs. The nucleic acid listed in SEQ ID NO:6 depicts the ITR in the "flip" orientation of the ITR. The nucleic acid listed in SEQ ID
15 NO:20 depicts the ITR in the "flop" orientation of the ITR. Minor modifications in an ITR of either orientation are those that will not interfere with the hairpin structure formed by the AAV4 ITR as described herein and known in the art. Furthermore, to be considered within the term "AAV4 ITRs" the nucleotide sequence must retain the Rep binding site described herein and exemplified in SEQ ID NO:6 and SEQ ID NO:20, *i.e.*,
20 it must retain one or both features described herein that distinguish the AAV4 ITR from the AAV2 ITR: (1) four (rather than three as in AAV2) "GAGC" repeats and (2) in the AAV4 ITR Rep binding site the fourth nucleotide in the first two "GAGC" repeats is a T rather than a C.

25 The promoter can be any desired promoter, selected by known considerations, such as the level of expression of a nucleic acid functionally linked to the promoter and the cell type in which the vector is to be used. Promoters can be an exogenous or an endogenous promoter. Promoters can include, for example, known strong promoters such as SV40 or the inducible metallothionein promoter, or an AAV promoter, such as
30 an AAV p5 promoter. Additional examples of promoters include promoters derived from actin genes, immunoglobulin genes, cytomegalovirus (CMV), adenovirus, bovine

papilloma virus, adenoviral promoters, such as the adenoviral major late promoter, an inducible heat shock promoter, respiratory syncytial virus, Rous sarcomas virus (RSV), etc. Specifically, the promoter can be AAV2 p5 promoter or AAV4 p5 promoter.

More specifically, the AAV4 p5 promoter can be about nucleotides 130 to 291 of SEQ

5 ID NO: 1. Additionally, the p5 promoter may be enhanced by nucleotides 1-130.

Furthermore, smaller fragments of p5 promoter that retain promoter activity can readily be determined by standard procedures including, for example, constructing a series of deletions in the p5 promoter, linking the deletion to a reporter gene, and determining whether the reporter gene is expressed, *i.e.*, transcribed and/or translated.

10

It should be recognized that the nucleotide and amino acid sequences set forth herein may contain minor sequencing errors. Such errors in the nucleotide sequences can be corrected, for example, by using the hybridization procedure described above with various probes derived from the described sequences such that the coding sequence
15 can be reisolated and resequenced. The corresponding amino acid sequence can then be corrected accordingly.

The AAV4 vector can further comprise an exogenous nucleic acid functionally
20 linked to the promoter. By "heterologous nucleic acid" is meant that any heterologous or exogenous nucleic acid can be inserted into the vector for transfer into a cell, tissue or organism. The nucleic acid can encode a polypeptide or protein or an antisense RNA, for example. By "functionally linked" is meant such that the promoter can promote expression of the heterologous nucleic acid, as is known in the art, such as
25 appropriate orientation of the promoter relative to the heterologous nucleic acid. Furthermore, the heterologous nucleic acid preferably has all appropriate sequences for expression of the nucleic acid, as known in the art, to functionally encode, *i.e.*, allow the nucleic acid to be expressed. The nucleic acid can include, for example, expression control sequences, such as an enhancer, and necessary information processing sites, such
30 as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences.

The heterologous nucleic acid can encode beneficial proteins that replace missing or defective proteins required by the subject into which the vector is transferred or can encode a cytotoxic polypeptide that can be directed, *e.g.*, to cancer cells or other cells whose death would be beneficial to the subject. The heterologous nucleic acid can also
5 encode antisense RNAs that can bind to, and thereby inactivate, mRNAs made by the subject that encode harmful proteins. In one embodiment, antisense polynucleotides can be produced from a heterologous expression cassette in an AAV4 viral construct where the expression cassette contains a sequence that promotes cell-type specific expression (Wirak *et al.*, *EMBO* 10:289 (1991)). For general methods relating to
10 antisense polynucleotides, see *Antisense RNA and DNA*, D. A. Melton, Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1988).

Examples of heterologous nucleic acids which can be administered to a cell or subject as part of the present AAV4 vector can include, but are not limited to the
15 following: nucleic acids encoding therapeutic agents, such as tumor necrosis factors (TNF), such as TNF- α ; interferons, such as interferon- α , interferon- β , and interferon- γ ; interleukins, such as IL-1, IL-1 β , and ILs -2 through -14; GM-CSF; adenosine deaminase; cellular growth factors, such as lymphokines; soluble CD4; Factor VIII; Factor IX; T-cell receptors; LDL receptor; ApoE; ApoC; alpha-1 antitrypsin; ornithine
20 transcarbamylase (OTC); cystic fibrosis transmembrane receptor (CFTR); insulin; Fc receptors for antigen binding domains of antibodies, such as immunoglobulins; and antisense sequences which inhibit viral replication, such as antisense sequences which inhibit replication of hepatitis B or hepatitis non-A, non-B virus. The nucleic acid is chosen considering several factors, including the cell to be transfected. Where the target
25 cell is a blood cell, for example, particularly useful nucleic acids to use are those which allow the blood cells to exert a therapeutic effect, such as a gene encoding a clotting factor for use in treatment of hemophilia. Furthermore, the nucleic acid can encode more than one gene product, limited only, if the nucleic acid is to be packaged in a capsid, by the size of nucleic acid that can be packaged.

Furthermore, suitable nucleic acids can include those that, when transferred into a primary cell, such as a blood cell, cause the transferred cell to target a site in the body where that cell's presence would be beneficial. For example, blood cells such as TIL cells can be modified, such as by transfer into the cell of a Fab portion of a monoclonal antibody, to recognize a selected antigen. Another example would be to introduce a nucleic acid that would target a therapeutic blood cell to tumor cells. Nucleic acids useful in treating cancer cells include those encoding chemotactic factors which cause an inflammatory response at a specific site, thereby having a therapeutic effect.

Cells, particularly blood cells, having such nucleic acids transferred into them can be useful in a variety of diseases, syndromes and conditions. For example, suitable nucleic acids include nucleic acids encoding soluble CD4, used in the treatment of AIDS and α -antitrypsin, used in the treatment of emphysema caused by α -antitrypsin deficiency. Other diseases, syndromes and conditions in which such cells can be useful include, for example, adenosine deaminase deficiency, sickle cell deficiency, brain disorders such as Alzheimer's disease, thalassemia, hemophilia, diabetes, phenylketonuria, growth disorders and heart diseases, such as those caused by alterations in cholesterol metabolism, and defects of the immune system.

As another example, hepatocytes can be transfected with the present vectors having useful nucleic acids to treat liver disease. For example, a nucleic acid encoding OTC can be used to transfect hepatocytes (*ex vivo* and returned to the liver or *in vivo*) to treat congenital hyperammonemia, caused by an inherited deficiency in OTC. Another example is to use a nucleic acid encoding LDL to target hepatocytes *ex vivo* or *in vivo* to treat inherited LDL receptor deficiency. Such transfected hepatocytes can also be used to treat acquired infectious diseases, such as diseases resulting from a viral infection. For example, transduced hepatocyte precursors can be used to treat viral hepatitis, such as hepatitis B and non-A, non-B hepatitis, for example by transducing the hepatocyte precursor with a nucleic acid encoding an antisense RNA that inhibits viral replication. Another example includes transferring a vector of the present invention

having a nucleic acid encoding a protein, such as α -interferon, which can confer resistance to the hepatitis virus.

For a procedure using transfected hepatocytes or hepatocyte precursors, hepatocyte precursors having a vector of the present invention transferred in can be grown in tissue culture, removed from the tissue culture vessel, and introduced to the body, such as by a surgical method. In this example, the tissue would be placed directly into the liver, or into the body cavity in proximity to the liver, as in a transplant or graft. Alternatively, the cells can simply be directly injected into the liver, into the portal circulatory system, or into the spleen, from which the cells can be transported to the liver via the circulatory system. Furthermore, the cells can be attached to a support, such as microcarrier beads, which can then be introduced, such as by injection, into the peritoneal cavity. Once the cells are in the liver, by whatever means, the cells can then express the nucleic acid and/or differentiate into mature hepatocytes which can express the nucleic acid.

The present invention also contemplates any unique fragment of these AAV4 nucleic acids, including the AAV4 nucleic acids set forth in SEQ ID NOs: 1, 3, 5, 6, 7, 12-15, 17 and 19. To be unique, the fragment must be of sufficient size to distinguish it from other known sequences, most readily determined by comparing any nucleic acid fragment to the nucleotide sequences of nucleic acids in computer databases, such as GenBank. Such comparative searches are standard in the art. Typically, a unique fragment useful as a primer or probe will be at least about 8 or 10 to about 20 or 25 nucleotides in length, depending upon the specific nucleotide content of the sequence. Additionally, fragments can be, for example, at least about 30, 40, 50, 75, 100, 200 or 500 nucleotides in length. The nucleic acid can be single or double stranded, depending upon the purpose for which it is intended.

The present invention further provides an AAV4 capsid protein. In particular, the present invention provides not only a polypeptide comprising all three AAV4 coat proteins, *i.e.*, VP1, VP2 and VP3, but also a polypeptide comprising each AAV4 coat

protein individually. Thus an AAV4 particle comprising an AAV4 capsid protein comprises at least one AAV4 coat protein VP1, VP2 or VP3. An AAV4 particle comprising an AAV4 capsid protein can be utilized to deliver a nucleic acid vector to a cell, tissue or subject. For example, the herein described AAV4 vectors can be
5 encapsulated in an AAV4 particle and utilized in a gene delivery method. Furthermore, other viral nucleic acids can be encapsidated in the AAV4 particle and utilized in such delivery methods. For example, an AAV2 vector can be encapsidated in an AAV4 particle and administered. Furthermore, a chimeric capsid protein incorporating both AAV2 and AAV4 sequences can be generated, by standard cloning methods, selecting
10 regions from each protein as desired. For example, particularly antigenic regions of the AAV2 capsid protein can be replaced with the corresponding region of the AAV4 capsid protein.

The herein described AAV4 nucleic acid vector can be encapsidated in an AAV
15 particle. In particular, it can be encapsidated in an AAV1 particle, an AAV2 particle, an AAV3 particle, an AAV4 particle, or an AAV5 particle by standard methods using the appropriate capsid proteins in the encapsidation process, as long as the nucleic acid
vector fits within the size limitation of the particle utilized. The encapsidation process itself is standard in the art.

20

An AAV4 particle is a viral particle comprising an AAV4 capsid protein. An AAV4 capsid polypeptide encoding the entire VP1, VP2, and VP3 polypeptide can overall have at least about 63% homology to the polypeptide having the amino acid sequence encoded by nucleotides 2260-4464 set forth in SEQ ID NO:1 (AAV4 capsid
25 protein). The capsid protein can have about 70% homology, about 75% homology, 80% homology, 85% homology, 90% homology, 95% homology, 98% homology, 99% homology, or even 100% homology to the protein having the amino acid sequence encoded by nucleotides 2260-4464 set forth in SEQ ID NO:1. The particle can be a particle comprising both AAV4 and AAV2 capsid protein, *i.e.*, a chimeric protein.
30 Variations in the amino acid sequence of the AAV4 capsid protein are contemplated herein, as long as the resulting viral particle comprising the AAV4 capsid remains

antigenically or immunologically distinct from AAV2, as can be routinely determined by standard methods. Specifically, for example, ELISA and Western blots can be used to determine whether a viral particle is antigenically or immunologically distinct from AAV2. Furthermore, the AAV4 viral particle preferably retains tissue tropism
5 distinction from AAV2, such as that exemplified in the examples herein, though an AAV4 chimeric particle comprising at least one AAV4 coat protein may have a different tissue tropism from that of an AAV4 particle consisting only of AAV4 coat proteins.

10 The invention further provides an AAV4 particle containing, *i.e.*, encapsidating, a vector comprising a pair of AAV2 inverted terminal repeats. The nucleotide sequence of AAV2 ITRs is known in the art. Furthermore, the particle can be a particle comprising both AAV4 and AAV2 capsid protein, *i.e.*, a chimeric protein. The vector encapsidated in the particle can further comprise an exogenous nucleic acid inserted
15 between the inverted terminal repeats.

The present invention further provides an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:1 (AAV4 genome). This nucleic acid, or portions thereof, can be inserted into other vectors, such as plasmids, yeast artificial
20 chromosomes, or other viral vectors, if desired, by standard cloning methods. The present invention also provides an isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO:1. The nucleotides of SEQ ID NO:1 can have minor modifications and still be contemplated by the present invention. For example, modifications that do not alter the amino acid encoded by any given codon
25 (such as by modification of the third, "wobble," position in a codon) can readily be made, and such alterations are known in the art. Furthermore, modifications that cause a resulting neutral amino acid substitution of a similar amino acid can be made in a coding region of the genome. Additionally, modifications as described herein for the AAV4 components, such as the ITRs, the p5 promoter, etc. are contemplated in this
30 invention.

The present invention additionally provides an isolated nucleic acid that selectively hybridizes with an isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO:1 (AAV4 genome). The present invention further provides an isolated nucleic acid that selectively hybridizes with an isolated
5 nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:1 (AAV4 genome). By "selectively hybridizes" as used in the claims is meant a nucleic acid that specifically hybridizes to the particular target nucleic acid under sufficient stringency conditions to selectively hybridize to the target nucleic acid without significant background hybridization to a nucleic acid encoding an unrelated protein, and
10 particularly, without detectably hybridizing to AAV2. Thus, a nucleic acid that selectively hybridizes with a nucleic acid of the present invention will not selectively hybridize under stringent conditions with a nucleic acid encoding a different protein, and vice versa. Therefore, nucleic acids for use, for example, as primers and probes to detect or amplify the target nucleic acids are contemplated herein. Nucleic acid
15 fragments that selectively hybridize to any given nucleic acid can be used, *e.g.*, as primers and or probes for further hybridization or for amplification methods (*e.g.*, polymerase chain reaction (PCR), ligase chain reaction (LCR)). Additionally, for example, a primer or probe can be designed that selectively hybridizes with both AAV4 and a gene of interest carried within the AAV4 vector (*i.e.*, a chimeric nucleic acid).

20

Stringency of hybridization is controlled by both temperature and salt concentration of either or both of the hybridization and washing steps. Typically, the stringency of hybridization to achieve selective hybridization involves hybridization in high ionic strength solution (6X SSC or 6X SSPE) at a temperature that is about 12-
25 25°C below the T_m (the melting temperature at which half of the molecules dissociate from its partner) followed by washing at a combination of temperature and salt concentration chosen so that the washing temperature is about 5°C to 20°C below the T_m . The temperature and salt conditions are readily determined empirically in preliminary experiments in which samples of reference DNA immobilized on filters are hybridized to
30 a labeled nucleic acid of interest and then washed under conditions of different stringencies. Hybridization temperatures are typically higher for DNA-RNA and RNA-

RNA hybridizations. The washing temperatures can be used as described above to achieve selective stringency, as is known in the art. (Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989; Kunkel et al. *Methods Enzymol.* 1987:154:367, 1987). A
5 preferable stringent hybridization condition for a DNA:DNA hybridization can be at about 68°C (in aqueous solution) in 6X SSC or 6X SSPE followed by washing at 68°C.

Stringency of hybridization and washing, if desired, can be reduced accordingly as homology desired is decreased, and further, depending upon the G-C or A-T richness of any area wherein variability is searched for. Likewise, stringency of hybridization and
10 washing, if desired, can be increased accordingly as homology desired is increased, and further, depending upon the G-C or A-T richness of any area wherein high homology is desired, all as known in the art.

A nucleic acid that selectively hybridizes to any portion of the AAV4 genome is
15 contemplated herein. Therefore, a nucleic acid that selectively hybridizes to AAV4 can be of longer length than the AAV4 genome, it can be about the same length as the AAV4 genome or it can be shorter than the AAV4 genome. The length of the nucleic acid is limited on the shorter end of the size range only by its specificity for hybridization to AAV4, *i.e.*, once it is too short, typically less than about 5 to 7 nucleotides in length,
20 it will no longer bind specifically to AAV4, but rather will hybridize to numerous background nucleic acids. Additionally contemplated by this invention is a nucleic acid that has a portion that specifically hybridizes to AAV4 and a portion that specifically hybridizes to a gene of interest inserted within AAV4.

25 The present invention further provides an isolated nucleic acid encoding an adeno-associated virus 4 Rep protein. The AAV4 Rep proteins are encoded by open reading frame (ORF) 1 of the AAV4 genome. The AAV4 Rep genes are exemplified by the nucleic acid set forth in SEQ ID NO:3 (AAV4 ORF1), and include a nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO:3 and a nucleic
30 acid comprising the nucleotide sequence set forth in SEQ ID NO:3. The present invention also includes a nucleic acid encoding the amino acid sequence set forth in SEQ

ID NO: 2 (polypeptide encoded by AAV4 ORF1). However, the present invention includes that the Rep genes nucleic acid can include any one, two, three, or four of the four Rep proteins, in any order, in such a nucleic acid. Furthermore, minor modifications are contemplated in the nucleic acid, such as silent mutations in the coding sequences, mutations that make neutral or conservative changes in the encoded amino acid sequence, and mutations in regulatory regions that do not disrupt the expression of the gene. Examples of other minor modifications are known in the art. Further modifications can be made in the nucleic acid, such as to disrupt or alter expression of one or more of the Rep proteins in order to, for example, determine the effect of such a disruption; such as to mutate one or more of the Rep proteins to determine the resulting effect, etc. However, in general, a modified nucleic acid encoding all four Rep proteins will have at least about 90%, about 93%, about 95%, about 98% or 100% homology to the sequence set forth in SEQ ID NO:3, and the Rep polypeptide encoded therein will have overall about 93%, about 95%, about 98%, about 99% or 100% homology with the amino acid sequence set forth in SEQ ID NO:2.

The present invention also provides an isolated nucleic acid that selectively hybridizes with a nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO:3 and an isolated nucleic acid that selectively hybridizes with a nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:3. "Selectively hybridizing" is defined elsewhere herein.

The present invention also provides each individual AAV4 Rep protein and the nucleic acid encoding each. Thus the present invention provides the nucleic acid encoding a Rep 40 protein, and in particular an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:12, an isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO:12, and a nucleic acid encoding the adeno-associated virus 4 Rep protein having the amino acid sequence set forth in SEQ ID NO:8. The present invention also provides the nucleic acid encoding a Rep 52 protein, and in particular an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:13, an isolated nucleic acid consisting essentially of

the nucleotide sequence set forth in SEQ ID NO:13, and a nucleic acid encoding the adeno-associated virus 4 Rep protein having the amino acid sequence set forth in SEQ ID NO:9. The present invention further provides the nucleic acid encoding a Rep 68 protein, and in particular an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:14, an isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO:14, and a nucleic acid encoding the adeno-associated virus 4 Rep protein having the amino acid sequence set forth in SEQ ID NO:10. And, further, the present invention provides the nucleic acid encoding a Rep 78 protein, and in particular an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:15, an isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO:15, and a nucleic acid encoding the adeno-associated virus 4 Rep protein having the amino acid sequence set forth in SEQ ID NO:11. As described elsewhere herein, these nucleic acids can have minor modifications, including silent nucleotide substitutions, mutations causing neutral amino acid substitutions in the encoded proteins, and mutations in control regions that do not or minimally affect the encoded amino acid sequence.

The present invention further provides a nucleic acid encoding the entire AAV4 Capsid polypeptide. Specifically, the present invention provides a nucleic acid having the nucleotide sequence set for the nucleotides 2260-4464 of SEQ ID NO:1. Furthermore, the present invention provides a nucleic acid encoding each of the three AAV4 coat proteins, VP1, VP2, and VP3. Thus, the present invention provides a nucleic acid encoding AAV4 VP1, a nucleic acid encoding AAV4 VP2, and a nucleic acid encoding AAV4 VP3. Thus, the present invention provides a nucleic acid encoding the amino acid sequence set forth in SEQ ID NO:4 (VP1); a nucleic acid encoding the amino acid sequence set forth in SEQ ID NO:16 (VP2), and a nucleic acid encoding the amino acid sequence set forth in SEQ ID NO:18 (VP3). The present invention also specifically provides a nucleic acid comprising SEQ ID NO:5 (VP1 gene); a nucleic acid comprising SEQ ID NO:17 (VP2 gene); and a nucleic acid comprising SEQ ID NO:19 (VP3 gene). The present invention also specifically provides a nucleic acid consisting essentially of SEQ ID NO:5 (VP1 gene), a nucleic acid consisting essentially of SEQ ID

NO:17 (VP2 gene), and a nucleic acid consisting essentially of SEQ ID NO:19 (VP3 gene). Furthermore, a nucleic acid encoding an AAV4 capsid protein VP1 is set forth as nucleotides 2157-4361 of SEQ ID NO:1; a nucleic acid encoding an AAV4 capsid protein VP2 is set forth as nucleotides 2565-4361 of SEQ ID NO:1; and a nucleic acid
5 encoding an AAV4 capsid protein VP3 is set forth as nucleotides 2745-4361 of SEQ ID NO:1. Minor modifications in the nucleotide sequences encoding the capsid, or coat, proteins are contemplated, as described above for other AAV4 nucleic acids.

The present invention also provides a cell containing one or more of the herein
10 described nucleic acids, such as the AAV4 genome, AAV4 ORF1 and ORF2, each AAV4 Rep protein gene, and each AAV4 capsid protein gene. Such a cell can be any desired cell and can be selected based upon the use intended. For example, cells can include human HeLa cells, cos cells, other human and mammalian cells and cell lines. Primary cultures as well as established cultures and cell lines can be used. Nucleic acids
15 of the present invention can be delivered into cells by any selected means, in particular depending upon the target cells. Many delivery means are well-known in the art. For example, electroporation, calcium phosphate precipitation, microinjection, cationic or anionic liposomes, and liposomes-in-combination with a nuclear localization signal
peptide for delivery to the nucleus can be utilized, as is known in the art. Additionally, if
20 in a viral particle, the cells can simply be transfected with the particle by standard means known in the art for AAV transfection.

The term "polypeptide" as used herein refers to a polymer of amino acids and includes full-length proteins and fragments thereof. Thus, "protein," polypeptide," and
25 "peptide" are often used interchangeably herein. Substitutions can be selected by known parameters to be neutral (*see, e.g.*, Robinson WE Jr, and Mitchell WM., AIDS 4:S151-S162 (1990)). As will be appreciated by those skilled in the art, the invention also includes those polypeptides having slight variations in amino acid sequences or other properties. Such variations may arise naturally as allelic variations (*e.g.*, due to
30 genetic polymorphism) or may be produced by human intervention (*e.g.*, by mutagenesis of cloned DNA sequences), such as induced point, deletion, insertion and substitution

mutants. Minor changes in amino acid sequence are generally preferred, such as conservative amino acid replacements, small internal deletions or insertions, and additions or deletions at the ends of the molecules. Substitutions may be designed based on, for example, the model of Dayhoff, *et al.* (in *Atlas of Protein Sequence and Structure* 1978, Nat'l Biomed. Res. Found., Washington, D.C.). These modifications can result in changes in the amino acid sequence, provide silent mutations, modify a restriction site, or provide other specific mutations.

A polypeptide of the present invention can be readily obtained by any of several means. For example, polypeptide of interest can be synthesized mechanically by standard methods. Additionally, the coding regions of the genes can be expressed and the resulting polypeptide isolated by standard methods. Furthermore, an antibody specific for the resulting polypeptide can be raised by standard methods (see, *e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1988), and the protein can be isolated from a cell expressing the nucleic acid encoding the polypeptide by selective hybridization with the antibody. This protein can be purified to the extent desired by standard methods of protein purification (see, *e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989).

Typically, to be unique, a polypeptide fragment of the present invention will be at least about 5 amino acids in length; however, unique fragments can be 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids in length. A unique polypeptide will typically comprise such a unique fragment; however, a unique polypeptide can also be determined by its overall homology. A unique polypeptide can be 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids in length. Uniqueness of a polypeptide fragment can readily be determined by standard methods such as searches of computer databases of known peptide or nucleic acid sequences or by hybridization studies to the nucleic acid encoding the protein or to the protein itself, as known in the art.

The present invention provides an isolated AAV4 Rep protein. AAV4 Rep polypeptide is encoded by ORF1 of AAV4. Specifically, the present invention provides an AAV4 Rep polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2, or a unique fragment thereof. The present invention also provides an AAV4 Rep polypeptide consisting essentially of the amino acid sequence set forth in SEQ ID NO:2, or a unique fragment thereof. Additionally, nucleotides 291-2306 of the AAV4 genome, which genome is set forth in SEQ ID NO:1, encode the AAV4 Rep polypeptide. The present invention also provides each AAV4 Rep protein. Thus the present invention provides AAV4 Rep 40, or a unique fragment thereof. The present invention particularly provides Rep 40 having the amino acid sequence set forth in SEQ ID NO:8. The present invention provides AAV4 Rep 52, or a unique fragment thereof. The present invention particularly provides Rep 52 having the amino acid sequence set forth in SEQ ID NO:9. The present invention provides AAV4 Rep 68, or a unique fragment thereof. The present invention particularly provides Rep 68 having the amino acid sequence set forth in SEQ ID NO:10. The present invention provides AAV4 Rep 78, or a unique fragment thereof. ~~The present invention particularly provides Rep 78 having the amino acid sequence set forth in SEQ ID NO:11.~~ By "unique fragment thereof" is meant any smaller polypeptide fragment encoded by AAV rep gene that is of sufficient length to be unique to the Rep polypeptide. Substitutions and modifications of the amino acid sequence can be made as described above and, further, can include protein processing modifications, such as glycosylation, to the polypeptide. However, a polypeptide including all four Rep proteins will encode a polypeptide having at least about 91% overall homology to the sequence set forth in SEQ ID NO:2, and it can have about 93%, about 95%, about 98%, about 99% or 100% homology with the amino acid sequence set forth in SEQ ID NO:2.

The present invention further provides an AAV4 Capsid polypeptide or a unique fragment thereof. AAV4 capsid polypeptide is encoded by ORF 2 of AAV4. Specifically, the present invention provides an AAV4 Capsid protein comprising the

amino acid sequence encoded by nucleotides 2260-4464 of the nucleotide sequence set forth in SEQ ID NO:1, or a unique fragment of such protein. The present invention also provides an AAV4 Capsid protein consisting essentially of the amino acid sequence encoded by nucleotides 2260-4464 of the nucleotide sequence set forth in SEQ ID

5 NO:1, or a unique fragment of such protein. The present invention further provides the individual AAV4 coat proteins, VP1, VP2 and VP3. Thus, the present invention provides an isolated polypeptide having the amino acid sequence set forth in SEQ ID NO:4 (VP1). The present invention additionally provides an isolated polypeptide having the amino acid sequence set forth in SEQ ID NO:16 (VP2). The present

10 invention also provides an isolated polypeptide having the amino acid sequence set forth in SEQ ID NO:18 (VP3). By "unique fragment thereof" is meant any smaller polypeptide fragment encoded by any AAV4 capsid gene that is of sufficient length to be unique to the AAV4 Capsid protein. Substitutions and modifications of the amino acid sequence can be made as described above and, further, can include protein processing

15 modifications, such as glycosylation, to the polypeptide. However, an AAV4 Capsid polypeptide including all three coat proteins will have at least about 63% overall homology to the polypeptide encoded by nucleotides 2260-4464 of the sequence set forth in SEQ ID NO: 1. The protein can have about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95% or even 100% homology to the amino

20 acid sequence encoded by the nucleotides 2260-4464 of the sequence set forth in SEQ ID NO:4. An AAV4 VP2 polypeptide can have at least about 58%, about 60%, about 70%, about 80%, about 90% about 95% or about 100% homology to the amino acid sequence set forth in SEQ ID NO:16. An AAV4 VP3 polypeptide can have at least about 60%, about 70%, about 80%, about 90% about 95% or about 100% homology to

25 the amino acid sequence set forth in SEQ ID NO:18.

The present invention further provides an isolated antibody that specifically binds AAV4 Rep protein. Also provided is an isolated antibody that specifically binds the AAV4 Rep protein having the amino acid sequence set forth in SEQ ID NO:2, or that

30 specifically binds a unique fragment thereof. Clearly, any given antibody can recognize and bind one of a number of possible epitopes present in the polypeptide; thus only a

unique portion of a polypeptide (having the epitope) may need to be present in an assay to determine if the antibody specifically binds the polypeptide.

The present invention additionally provides an isolated antibody that specifically binds any adeno-associated virus 4 Capsid protein or the polypeptide comprising all three AAV4 coat proteins. Also provided is an isolated antibody that specifically binds the AAV4 Capsid protein having the amino acid sequence set forth in SEQ ID NO:4, or that specifically binds a unique fragment thereof. The present invention further provides an isolated antibody that specifically binds the AAV4 Capsid protein having the amino acid sequence set forth in SEQ ID NO:16, or that specifically binds a unique fragment thereof. The invention additionally provides an isolated antibody that specifically binds the AAV4 Capsid protein having the amino acid sequence set forth in SEQ ID NO:18, or that specifically binds a unique fragment thereof. Again, any given antibody can recognize and bind one of a number of possible epitopes present in the polypeptide; thus only a unique portion of a polypeptide (having the epitope) may need to be present in an assay to determine if the antibody specifically binds the polypeptide.

The antibody can be a component of a composition that comprises an antibody that specifically binds the AAV4 protein. The composition can further comprise, *e.g.*, serum, serum-free medium, or a pharmaceutically acceptable carrier such as physiological saline, etc..

By "an antibody that specifically binds" an AAV4 polypeptide or protein is meant an antibody that selectively binds to an epitope on any portion of the AAV4 peptide such that the antibody selectively binds to the AAV4 polypeptide, *i.e.*, such that the antibody binds specifically to the corresponding AAV4 polypeptide without significant background. Specific binding by an antibody further means that the antibody can be used to selectively remove the target polypeptide from a sample comprising the polypeptide or and can readily be determined by radioimmuno assay (RIA), bioassay, or enzyme-linked immunosorbant (ELISA) technology. An ELISA method effective for the detection of the specific antibody-antigen binding can, for example, be as follows:

(1) bind the antibody to a substrate; (2) contact the bound antibody with a sample containing the antigen; (3) contact the above with a secondary antibody bound to a detectable moiety (e.g., horseradish peroxidase enzyme or alkaline phosphatase enzyme); (4) contact the above with the substrate for the enzyme; (5) contact the above
5 with a color reagent; (6) observe the color change.

An antibody can include antibody fragments such as Fab fragments which retain the binding activity. Antibodies can be made as described in, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring
10 Harbor, New York (1988). Briefly, purified antigen can be injected into an animal in an amount and in intervals sufficient to elicit an immune response. Antibodies can either be purified directly, or spleen cells can be obtained from the animal. The cells are then fused with an immortal cell line and screened for antibody secretion. Individual hybridomas are then propagated as individual clones serving as a source for a particular
15 monoclonal antibody.

The present invention additionally provides a method of screening a cell for infectivity by AAV4 comprising contacting the cell with AAV4 and detecting the presence of AAV4 in the cells. AAV4 particles can be detected using any standard
20 physical or biochemical methods. For example, physical methods that can be used for this detection include 1) polymerase chain reaction (PCR) for viral DNA or RNA, 2) direct hybridization with labeled probes, 3) antibody directed against the viral structural or non- structural proteins. Catalytic methods of viral detection include, but are not limited to, detection of site and strand specific DNA nicking activity of Rep proteins or
25 replication of an AAV origin- containing substrate. Additional detection methods are outlined in Fields, *Virology*, Raven Press, New York, New York. 1996.

For screening a cell for infectivity by AAV4 wherein the presence of AAV4 in the cells is determined by nucleic acid hybridization methods, a nucleic acid probe for
30 such detection can comprise, for example, a unique fragment of any of the AAV4 nucleic acids provided herein. The uniqueness of any nucleic acid probe can readily be

determined as described herein for unique nucleic acids. The nucleic acid can be, for example, the nucleic acid whose nucleotide sequence is set forth in SEQ ID NO: 1, 3, 5, 6, 7, 12, 13, 14, 15, 17 or 19, or a unique fragment thereof.

5 The present invention includes a method of determining the suitability of an AAV4 vector for administration to a subject comprising administering to an antibody-containing sample from the subject an antigenic fragment of an isolated AAV4 capsid protein, and detecting an antibody-antigen reaction in the sample, the presence of a reaction indicating the AAV4 vector to be unsuitable for use in the subject. The AAV4
10 capsid protein from which an antigenic fragment is selected can have the amino acid sequence set forth in SEQ ID NO:4. An immunogenic fragment of an isolated AAV4 capsid protein can also be used in these methods. The AAV4 capsid protein from which an antigenic fragment is selected can have the amino acid sequence set forth in SEQ ID NO:17. The AAV4 capsid protein from which an antigenic fragment is selected can
15 have the amino acid sequence set forth in SEQ ID NO:19.

Alternatively, or additionally, an antigenic fragment of an isolated AAV4 Rep
~~protein can be utilized in this determination method. An immunogenic fragment of an~~
isolated AAV4 Rep protein can also be used in these methods. Thus the present
20 invention further provides a method of determining the suitability of an AAV4 vector for administration to a subject comprising administering to an antibody-containing sample from the subject an antigenic fragment of an AAV4 Rep protein and detecting an antibody-antigen reaction in the sample, the presence of a reaction indicating the AAV4 vector to be unsuitable for use in the subject. The AAV4 Rep protein from which an
25 antigenic fragment is selected can have the amino acid sequence set forth in SEQ ID NO:2. The AAV4 Rep protein from which an antigenic fragment is selected can have the amino acid sequence set forth in SEQ ID NO:8. The AAV4 Rep protein from which an antigenic fragment is selected can have the amino acid sequence set forth in SEQ ID NO:9. The AAV4 Rep protein from which an antigenic fragment is selected can have
30 the amino acid sequence set forth in SEQ ID NO:10. The AAV4 Rep protein from

which an antigenic fragment is selected can have the amino acid sequence set forth in SEQ ID NO:11.

An antigenic or immunoreactive fragment is typically an amino acid sequence of at least about 5 consecutive amino acids, and it can be derived from the AAV4 polypeptide amino acid sequence. An antigenic fragment is any fragment unique to the AAV4 protein, as described herein, against which an AAV4-specific antibody can be raised, by standard methods. Thus, the resulting antibody-antigen reaction should be specific for AAV4.

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The AAV4 polypeptide fragments can be analyzed to determine their antigenicity, immunogenicity and/or specificity. Briefly, various concentrations of a putative immunogenically specific fragment are prepared and administered to a subject and the immunological response (e.g., the production of antibodies or cell mediated immunity) of an animal to each concentration is determined. The amounts of antigen administered depend on the subject, e.g. a human, rabbit or a guinea pig, the condition of the subject, the size of the subject, etc. Thereafter an animal so inoculated with the antigen can be exposed to the AAV4 viral particle or AAV4 protein to test the immunoreactivity or the antigenicity of the specific immunogenic fragment. The specificity of a putative antigenic or immunogenic fragment can be ascertained by testing sera, other fluids or lymphocytes from the inoculated animal for cross reactivity with other closely related viruses, such as AAV1, AAV2, AAV3 and AAV5.

As will be recognized by those skilled in the art, numerous types of immunoassays are available for use in the present invention to detect binding between an antibody and an AAV4 polypeptide of this invention. For instance, direct and indirect binding assays, competitive assays, sandwich assays, and the like, as are generally described in, e.g., U.S. Pat. Nos. 4,642,285; 4,376,110; 4,016,043; 3,879,262; 3,852,157; 3,850,752; 3,839,153; 3,791,932; and Harlow and Lane, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, N.Y. (1988). For example, enzyme immunoassays such as immunofluorescence assays (IFA), enzyme linked

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immunosorbent assays (ELISA) and immunoblotting can be readily adapted to accomplish the detection of the antibody. An ELISA method effective for the detection of the antibody bound to the antigen can, for example, be as follows: (1) bind the antigen to a substrate; (2) contact the bound antigen with a fluid or tissue sample containing the antibody; (3) contact the above with a secondary antibody specific for the antigen and bound to a detectable moiety (e.g., horseradish peroxidase enzyme or alkaline phosphatase enzyme); (4) contact the above with the substrate for the enzyme; (5) contact the above with a color reagent; (6) observe color change.

10 The antibody-containing sample of this method can comprise any biological sample which would contain the antibody or a cell containing the antibody, such as blood, plasma, serum, bone marrow, saliva and urine.

 By the "suitability of an AAV4 vector for administration to a subject" is meant a
15 determination of whether the AAV4 vector will elicit a neutralizing immune response upon administration to a particular subject. A vector that does not elicit a significant immune response is a potentially suitable vector, whereas a vector that elicits a significant, neutralizing immune response is thus indicated to be unsuitable for use in
that subject. Significance of any detectable immune response is a standard parameter
20 understood by the skilled artisan in the field. For example, one can incubate the subject's serum with the virus, then determine whether that virus retains its ability to transduce cells in culture. If such virus cannot transduce cells in culture, the vector likely has elicited a significant immune response.

25 The present method further provides a method of delivering a nucleic acid to a cell comprising administering to the cell an AAV4 particle containing a vector comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, thereby delivering the nucleic acid to the cell. Administration to the cell can be accomplished by any means, including simply contacting the particle, optionally
30 contained in a desired liquid such as tissue culture medium, or a buffered saline solution, with the cells. The particle can be allowed to remain in contact with the cells for any

desired length of time, and typically the particle is administered and allowed to remain indefinitely. For such *in vitro* methods, the virus can be administered to the cell by standard viral transduction methods, as known in the art and as exemplified herein. Titers of virus to administer can vary, particularly depending upon the cell type, but will be typical of that used for AAV transduction in general. Additionally the titers used to transduce the particular cells in the present examples can be utilized. The cells can include any desired cell, such as the following cells and cells derived from the following tissues, in humans as well as other mammals, such as primates, horse, sheep, goat, pig, dog, rat, and mouse: Adipocytes, Adenocyte, Adrenal cortex, Amnion, Aorta, Ascites, Astrocyte, Bladder, Bone, Bone marrow, Brain, Breast, Bronchus, Cardiac muscle, Cecum, Cervix, Chorion, Colon, Conjunctiva, Connective tissue, Cornea, Dermis, Duodenum, Endometrium, Endothelium, Epithelial tissue, Epidermis, Esophagus, Eye, Fascia, Fibroblasts, Foreskin, Gastric, Glial cells, Glioblast, Gonad, Hepatic cells, Histocyte, Ileum, Intestine, small Intestine, Jejunum, Keratinocytes, Kidney, Larynx, Leukocytes, Lipocyte, Liver, Lung, Lymph node, Lymphoblast, Lymphocytes, Macrophages, Mammary alveolar nodule, Mammary gland, Mastocyte, Maxilla, Melanocytes, Monocytes, Mouth, Myelin, Nervous tissue, Neuroblast, Neurons, Neuroglia, Osteoblasts, Osteogenic cells, Ovary, Palate, Pancreas, Papilloma, Peritoneum, Pituicytes, Pharynx, Placenta, Plasma cells, Pleura, Prostate, Rectum, Salivary gland, Skeletal muscle, Skin, Smooth muscle, Somatic, Spleen, Squamous, Stomach, Submandibular gland, Submaxillary gland, Synoviocytes, Testis, Thymus, Thyroid, Trabeculae, Trachea, Turbinate, Umbilical cord, Ureter, and Uterus.

The AAV inverted terminal repeats in the vector for the herein described delivery methods can be AAV4 inverted terminal repeats. Specifically, they can comprise the nucleic acid whose nucleotide sequence is set forth in SEQ ID NO:6 or the nucleic acid whose nucleotide sequence is set forth in SEQ ID NO:20, or any fragment thereof demonstrated to have ITR functioning. The ITRs can also consist essentially of the nucleic acid whose nucleotide sequence is set forth in SEQ ID NO:6 or the nucleic acid whose nucleotide sequence is set forth in SEQ ID NO:20. Furthermore, the AAV inverted terminal repeats in the vector for the herein described nucleic acid delivery

methods can also comprise AAV2 inverted terminal repeats. Additionally, the AAV inverted terminal repeats in the vector for this delivery method can also consist essentially of AAV2 inverted terminal repeats.

5 The present invention also includes a method of delivering a nucleic acid to a subject comprising administering to a cell from the subject an AAV4 particle comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, and returning the cell to the subject, thereby delivering the nucleic acid to the subject. The AAV ITRs can be any AAV ITRs, including AAV4 ITRs and AAV2 ITRs. For such an *ex vivo*
10 administration, cells are isolated from a subject by standard means according to the cell type and placed in appropriate culture medium, again according to cell type (*see, e.g.*, ATCC catalog). Viral particles are then contacted with the cells as described above, and the virus is allowed to transfect the cells. Cells can then be transplanted back into the subject's body, again by means standard for the cell type and tissue (*e. g.*, in general,
15 U.S. Patent No. 5,399,346; for neural cells, Dunnett, S.B. and Björklund, A., eds., *Transplantation: Neural Transplantation-A Practical Approach*, Oxford University Press, Oxford (1992)). If desired, prior to transplantation, the cells can be studied for
~~degree of transfection by the virus, by known detection means and as described herein.~~
Cells for *ex vivo* transfection followed by transplantation into a subject can be selected
20 from those listed above, or can be any other selected cell. Preferably, a selected cell type is examined for its capability to be transfected by AAV4. Preferably, the selected cell will be a cell readily transduced with AAV4 particles; however, depending upon the application, even cells with relatively low transduction efficiencies can be useful, particularly if the cell is from a tissue or organ in which even production of a small
25 amount of the protein or antisense RNA encoded by the vector will be beneficial to the subject.

The present invention further provides a method of delivering a nucleic acid to a cell in a subject comprising administering to the subject an AAV4 particle comprising
30 the nucleic acid inserted between a pair of AAV inverted terminal repeats, thereby delivering the nucleic acid to a cell in the subject. Administration can be an *ex vivo*

administration directly to a cell removed from a subject, such as any of the cells listed above, followed by replacement of the cell back into the subject, or administration can be *in vivo* administration to a cell in the subject. For *ex vivo* administration, cells are isolated from a subject by standard means according to the cell type and placed in
5 appropriate culture medium, again according to cell type (*see, e.g.,* ATCC catalog). Viral particles are then contacted with the cells as described above, and the virus is allowed to transfect the cells. Cells can then be transplanted back into the subject's body, again by means standard for the cell type and tissue (*e. g.,* for neural cells, Dunnett, S.B. and Björklund, A., eds., *Transplantation: Neural Transplantation-A*
10 *Practical Approach*, Oxford University Press, Oxford (1992)). If desired, prior to transplantation, the cells can be studied for degree of transfection by the virus, by known detection means and as described herein.

In vivo administration to a human subject or an animal model can be by any of
15 many standard means for administering viruses, depending upon the target organ, tissue or cell. Virus particles can be administered orally, parenterally (*e.g.,* intravenously), by intramuscular injection, by direct tissue or organ injection, by intraperitoneal injection, topically, transdermally, or the like. Viral nucleic acids (non-encapsidated) can be administered, *e.g.,* as a complex with cationic liposomes, or encapsulated in anionic
20 liposomes. Compositions can include various amounts of the selected viral particle or non-encapsidated viral nucleic acid in combination with a pharmaceutically acceptable carrier and, in addition, if desired, may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, diluents, etc. Parental administration, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as
25 liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Dosages will depend upon the mode of administration, the disease or condition to be treated, and the individual subject's condition, but will be that dosage typical for and used in administration of other AAV vectors, such as AAV2 vectors. Often a single dose can be sufficient; however, the dose
30 can be repeated if desirable.

The present invention further provides a method of delivering a nucleic acid to a cell in a subject having antibodies to AAV2 comprising administering to the subject an AAV4 particle comprising the nucleic acid, thereby delivering the nucleic acid to a cell in the subject. A subject that has antibodies to AAV2 can readily be determined by any
5 of several known means, such as contacting AAV2 protein(s) with an antibody-containing sample, such as blood, from a subject and detecting an antigen-antibody reaction in the sample. Delivery of the AAV4 particle can be by either *ex vivo* or *in vivo* administration as herein described. Thus, a subject who might have an adverse immunogenic reaction to a vector administered in an AAV2 viral particle can have a
10 desired nucleic acid delivered using an AAV4 particle. This delivery system can be particularly useful for subjects who have received therapy utilizing AAV2 particles in the past and have developed antibodies to AAV2. An AAV4 regimen can now be substituted to deliver the desired nucleic acid.

STATEMENT OF UTILITY

The present invention provides recombinant vectors based on AAV4. Such
5 vectors may be useful for transducing erythroid progenitor cells which is very inefficient
with AAV2 based vectors. In addition to transduction of other cell types, transduction
of erythroid cells would be useful for the treatment of cancer and genetic diseases which
can be corrected by bone marrow transplants using matched donors. Some examples of
this type of treatment include, but are not limited to, the introduction of a therapeutic
10 gene such as genes encoding interferons, interleukins, tumor necrosis factors, adenosine
deaminase, cellular growth factors such as lymphokines, blood coagulation factors such
as factor VIII and IX, cholesterol metabolism uptake and transport protein such as
EpoE and LDL receptor, and antisense sequences to inhibit viral replication of, for
example, hepatitis or HIV.

15

The present invention provides a vector comprising the AAV4 virus as well as
AAV4 viral particles. While AAV4 is similar to AAV2, the two viruses are found herein
to be physically and genetically distinct. These differences endow AAV4 with some
unique advantages which better suit it as a vector for gene therapy. For example, the wt
20 AAV4 genome is larger than AAV2, allowing for efficient encapsidation of a larger
recombinant genome. Furthermore, wt AAV4 particles have a greater buoyant density
than AAV2 particles and therefore are more easily separated from contaminating helper
virus and empty AAV particles than AAV2-based particles.

25 Furthermore, as shown herein, AAV4 capsid protein is distinct from AAV2
capsid protein and exhibits different tissue tropism. AAV2 and AAV4 are shown herein
to utilize distinct cellular receptors. AAV2 and AAV4 have been shown to be
serologically distinct and thus, in a gene therapy application, AAV4 would allow for
transduction of a patient who already possess neutralizing antibodies to AAV2 either as
30 a result of natural immunological defense or from prior exposure to AAV2 vectors.

The present invention is more particularly described in the following examples which are intended as illustrative only since numerous modifications and variations therein will be apparent to those skilled in the art.

5

EXAMPLES

To understand the nature of AAV4 virus and to determine its usefulness as a vector for gene transfer, it was cloned and sequenced.

10

Cell culture and virus propagation

Cos and HeLa cells were maintained as monolayer cultures in D10 medium (Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 100 ug/ml penicillin, 100 units/ml streptomycin and IX Fungizone as recommended by the manufacturer; (GIBCO, Gaithersburg, MD, USA) . All other cell types were grown under standard conditions which have been previously reported. AAV4 stocks were obtained from American Type Culture Collection # VR- 64 6.

Virus was produced as previously described for AAV2 using the Beta galactosidase vector plasmid and a helper plasmid containing the AAV4 Rep and Cap genes (9). The helper plasmid was constructed in such a way as not to allow any homologous sequence between the helper and vector plasmids. This step was taken to minimize the potential for wild-type (wt) particle formation by homologous recombination.

Virus was isolated from 5×10^7 cos cells by CsCl banding (9), and the distribution of Beta galactosidase genomes across the genome was determined by DNA dot blots of aliquots of gradient fractions. The majority of packaged genomes were found in fractions with a density of 1.43 which is similar to that reported for wt AAV4. This preparation of virus yielded 2.5×10^{11} particles or 5000 particles/producer cell. In comparison AAV2 isolated and CsCl banded from 8×10^7 cells yielded 1.2×10^{11} particles or 1500 particles/producer cell. Thus, typical yields of rAAV4 particles/producer cell were 3-5 fold greater than that of rAAV2 particles.

DNA Cloning and Sequencing and Analysis

In order to clone the genome of AAV4, viral lysate was amplified in cos cells
5 and then HeLa cells with the resulting viral particles isolated by CsCl banding. DNA dot
blots of aliquots of the gradient fractions indicated that peak genomes were contained in
fractions with a density of 1.41-1.45. This is very similar to the buoyant density
previously reported for AAV4 (29). Analysis of annealed DNA obtained from these
fractions indicated a major species of 4.8kb in length which upon restriction analysis
10 gave bands similar in size to those previously reported. Additional restriction analysis
indicated the presence of BssHII restriction sites near the ends of the DNA. Digestion
with BssHII yielded a 4.5kb fragment which was then cloned into Bluescript SKII+ and
two independent clones were sequenced.

The viral sequence is now available through Genbank, accession number
15 U89790. DNA sequence was determined using an ABI 373A automated sequencer and
the FS dye terminator chemistry. Both strands of the plasmids were sequenced and
confirmed by sequencing of a second clone. As further confirmation of the authenticity
of the sequence, bases 91-600 were PCR amplified from the original seed material and
directly sequenced. The sequence of this region, which contains a 56 base insertion
20 compared to AAV2 and 3, was found to be identical to that derived from the cloned
material. The ITR was cloned using Deep Vent Polymerase (New England Biolabs)
according to the manufactures instructions using the following primers, primer 1:
5'TCTAGTCTAGACTTGGCCACTCCCTCTCTGCGCGC(SEQ ID NO:21); primer 2:
51 AGGCCTTAAGAGCAGTCGTCCACCACCTTGTTCC (SEQ ID NO:22).
25 Cycling conditions were 97°C 20 sec, 65°C 30 sec, 75°C 1 min for 35 rounds.
Following the PCR reaction, the mixture was treated with XbaI and EcoRI
endonucleases and the amplified band purified by agarose gel electrophoresis. The
recovered DNA fragment was ligated into Bluescript SKII+ (Stratagene) and
transformed into competent Sure strain bacteria (Stratagene). The helper plasmid
30 (pSV40oriAAV₄₋₂) used for the production of recombinant virus, which contains the rep
and cap genes of AAV4, was produced by PCR with *Pfu* polymerase (Stratagene)

according to the manufactures instructions. The amplified sequence, nt 216-4440, was ligated into a plasmid that contains the SV40 origin of replication previously described (9, 10). Cycling conditions were 95°C 30 sec, 55°C 30 sec, 72°C 3 min for 20 rounds. The final clone was confirmed by sequencing. The β gal reporter vector has been
5 described previously (9, 10).

Sequencing of this fragment revealed two open reading frames (ORF) instead of only one as previously suggested. In addition to the previously identified Capsid ORF in the right-hand side of the genome, an additional ORF is present on the left-hand side. Computer analysis indicated that the left-hand ORF has a high degree of homology to
10 the Rep gene of AAV2. At the amino acid level the ORF is 90% identical to that of AAV2 with only 5% of the changes being non-conserved (SEQ ID NO:2). In contrast, the right ORF is only 62% identical at the amino acid level when compared to the corrected AAV2 sequence. While the internal start site of VP2 appears to be conserved, the start site for VP3 is in the middle of one of the two blocks of divergent sequence.
15 The second divergent block is in the middle of VP3. By using three dimensional structure analysis of the canine parvovirus and computer aided sequence comparisons, regions of AAV2 which might be exposed on the surface of the virus have been
identified. Comparison of the AAV2 and AAV4 sequences indicates that these regions
are not well conserved between the two viruses and suggests altered tissue tropism for
20 the two viruses.

Comparison of the p5 promoter region of the two viruses shows a high degree of conservation of known functional elements (SEQ ID NO:7). Initial work by Chang *et al.* identified two YY1 binding sites at -60 and +1 and a TATA Box at -30 which are all conserved between AAV2 and AAV4 (4). A binding site for the Rep has been identified
25 in the p5 promoter at -17 and is also conserved (24). The only divergence between the two viruses in this region appears to be in the sequence surrounding these elements. AAV4 also contains an additional 56 bases in this region between the p5 promoter and the TRS (nt 209-269). Based on its positioning in the viral genome and efficient use of the limited genome space, this sequence may possess some promoter activity or be
30 involved in rescue, replication or packaging of the virus.

The inverted terminal repeats were cloned by PCR using a probe derived from the terminal resolution site (TRS) of the BssHII fragment and a primer in the Rep ORF. The TRS is a sequence at the end of the stem of the ITR and the reverse complement of TRS sequence was contained within the BssHII fragment. The resulting fragments were
5 cloned and found to contain a number of sequence changes compared to AAV2. However, these changes were found to be complementary and did not affect the ability of this region to fold into a hairpin structure (Fig 2). While the TRS site was conserved between AAV2 and AAV4 the Rep binding site contained two alterations which expand the binding site from 3 GAGC repeats to 4. The first two repeats in AAV4 both contain
10 a T in the fourth position instead of a C. This type of repeat is present in the p5 promoter and is present in the consensus sequence that has been proposed for Rep binding (10) and its expansion may affect its affinity for Rep. Methylation interference data has suggested the importance of the CTTTG motif found at the tip of one
15 palindrome in Rep binding with the underlined T residues clearly affecting Rep binding to both the flip and flop forms. While most of this motif is conserved in AAV4 the middle T residue is changed to a C (33).

Hemagglutination assays

Hemagglutination was measured essentially as described previously (18). Serial
20 two fold dilutions of virus in Veronal-buffered saline were mixed with an equal volume of 0.4% human erythrocytes (type 0) in plastic U bottom 96 well plates. The reaction was complete after a 2 hr incubation at 8°C. HA units (HAU) are defined as the reciprocal of the dilution causing 50% hemagglutination.

The results show that both the wild type and recombinant AAV4 viruses can
25 hemagglutinate human red blood cells (RBCS) with HA titers of approximately 1024 HAU/ μ l and 512 HAU/ μ l respectively. No HA activity was detected with AAV type 3 or recombinant AAV type 2 as well as the helper adenovirus. If the temperature was raised to 22°C, HA activity decreased 32-fold. Comparison of the viral particle number per RBC at the end point dilution indicated that approximately 1-10 particles per RBC
30 were required for hemagglutination. This value is similar to that previously reported (18).

Tissue tropism analysis

The sequence divergence in the capsid proteins ORF which are predicted to be exposed on the surface of the virus may result in an altered binding specificity for AAV4 compared to AAV2. Very little is known about the tissue tropism of any dependovirus. While it had been shown to hemagglutinate human, guinea pig, and sheep erythrocytes, it is thought to be exclusively a simian virus (18). Therefore, to examine AAV4 tissue tropism and its species specificity, recombinant AAV4 particles which contained the gene for nuclear localized Beta galactosidase were constructed. Because of the similarity in genetic organization of AAV4 and AAV2, it was determined whether AAV4 particles could be constructed containing a recombinant genome. Furthermore, because of the structural similarities of the AAV type 2 and type 4 ITRs, a genome containing AAV2 ITRs which had been previously described was used.

Tissue tropism analysis 1. To study AAV transduction, a variety of cell lines were transduced with 5 fold serial dilutions of either recombinant AAV2 or AAV4 particles expressing the gene for nuclear localized Beta galactosidase activity (Table 1). Approximately 4×10^4 cells were exposed to virus in 0.5ml serum free media for 1 hour and then 1 ml of the appropriate complete media was added and the cells were incubated for 48-60 hours. The cells were then fixed and stained for β -galactosidase activity with 5-Bromo-4-Chloro-3-Indolyl- β -D-galactopyranoside (Xgal) (ICN Biomedicals) (36). Biological titers were determined by counting the number of positive cells in the different dilutions using a calibrated microscope ocular (3.1 mm^2) then multiplying by the area of the well and the dilution of the virus. Typically dilutions which gave 1-10 positive cells per field (100-1000 positive cells per 2cm well) were used for titer determination. Titers were determined by the average number of cells in a minimum of 10 fields/well.

To examine difference in tissue tropism, a number of cell lines were transduced with serial dilutions of either AAV4 or AAV2 and the biological titers determined. As shown in Table 1, when Cos cells were transduced with a similar number of viral particles, a similar level of transduction was observed with AAV2 and AAV4.

However, other cell lines exhibited differential transducibility by AAV2 or AAV4. Transduction of the human colon adenocarcinoma cell line SW480 with AAV2 was over 100 times higher than that obtained with AAV4. Furthermore, both vectors transduced SW1116, SW1463 and NIH3T3 cells relatively poorly.

5

TABLE 1

<u>Cell type</u>	<u>AAV2</u>	<u>AAV4</u>
Cos	4.5 X10 ⁷	1.9 X10 ⁷
10 SW 480	3.8 X10 ⁶	2.8 X10 ⁴
SW 1116	5.2 X10 ⁴	8 X10 ³
SW1463	8.8 X10 ⁴	8 X10 ³
SW620	8.8 X10 ⁴	ND
NIH 3T3	2 X10 ⁴	8X10 ³

15

Tissue tropism analysis 2.

A. Transduction of cells. Exponentially growing cells (2×10^4) were plated in each well of a 12 well plate and transduced with serial dilutions of virus in 200 μ l of medium for 1 hr. After this period, 800 μ l of additional medium was added and incubated for 48 hrs. The cells were then fixed and stained for β -galactosidase activity overnight with 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (Xgal) (ICN Biomedicals) (36). No endogenous β -galactosidase activity was visible after 24 hr incubation in Xgal solution. Infectious titers were determined by counting the number of positive cells in the different dilutions using a calibrated microscope ocular (diameter 3.1 mm²) then multiplying by the area of the well and the dilution of the virus. Titers were determined by the average number of cells in a minimum of 10 fields/well.

25

As shown in Table 2, cos cells transduced with equivalent amounts of rAAV2 and rAAV4 particles resulted in similar transduction levels. However, other cell lines exhibited differential transducibility. Transduction of the human colon adenocarcinoma cell line, SW480, with rAAV2 was 60 times higher than that obtained with rAAV4. Hela

30

and SW620 cells were also transduced more efficiently with rAAV2 than rAAV4. In contrast, transduction of primary rat brain cultures exhibited a greater transduction of glial and neuronal cells with rAAV4 compared to rAAV2. Because of the heterogeneous nature of the cell population in the rat brain cultures, only relative transduction efficiencies are reported (Table 2).

As a control for adenovirus contamination of the viral preparations cos and Hela cells were coinfectd with RAAV and adenovirus then stained after 24 hr. While the titer of rAAV2 increased in the presence of Ad in both cos and Hela, adenovirus only increased the titer in the cos cells transduced with rAAV4 and not the HeLa cells, suggesting the difference in transduction efficiencies is not the result of adenovirus contamination. Furthermore, both vectors transduced SW1116, SW1463, NIH3T3 and monkey fibroblasts FL2 cells very poorly. Thus AAV4 may utilize a cellular receptor distinct from that of AAV2.

15

TABLE 2

CELL TYPE	AAV2	AAV4
Primary Rat Brain	1	4.3± 0.7
cos	4.2X10 ⁷ ±4.6X10 ⁶	2.2X10 ⁷ ±2.5X10 ⁶
SW 480	7.75X10 ⁶ ±1.7X10 ⁶	1.3X10 ⁵ ±6.8X10 ⁴
Hela	2.1X10 ⁷ ±1X10 ⁶	1.3X10 ⁶ ±1X10 ⁵
SW620	1.2X10 ⁵ ±3.9X10 ⁴	4X10 ⁴
KLEB	1.2X10 ⁵ ±3.5X10 ⁴	9X10 ⁴ ±1.4X10 ⁴
HB	5.6X10 ⁵ ±2X10 ⁵	3.8X10 ⁴ ±1.8X10 ⁴
SW1116	5.2 X 10 ⁴	8 X 10 ³
SW1463	8.8 X 10 ⁴	8 X 10 ³
NIH 3T3	3 X 10 ³	2 X 10 ³

B. Competition assay. Cos cells were plated at 2×10^4 /well in 12 well plates 12-24 hrs prior to transduction. Cells were transduced with 0.5×10^7 particles of rAAV2 or rAAV4 (containing the LacZ gene) in 200 μ l of DMEM and increasing amounts of rAAV2 containing the gene for the human coagulation factor IX. Prior to transduction the CsCl was removed from the virus by dialysis against isotonic saline. After 1hr incubation with the recombinant virus the culture medium was supplemented with complete medium and allowed to incubate for 48-60 hrs. The cells were then stained and counted as described above.

AAV4 utilization of a cellular receptor distinct from that of AAV2 was further examined by cotransduction experiments with rAAV2 and rAAV4. Cos cells were transduced with an equal number of rAAV2 or rAAV4 particles containing the LacZ gene and increasing amounts of rAAV2 particles containing the human coagulation factor IX gene (rAAV2FIX). At a 72:1 ratio of rAAV2FIX:rAAV4LacZ only a two-fold effect on the level of rAAV4LacZ transduction was obtained (Fig 3). However this same ratio of rAAV2FIX:rAAV2LacZ reduced the transduction efficiency of rAAV2LacZ approximately 10 fold. Comparison of the 50% inhibition points for the two viruses indicated a 7 fold difference in sensitivity.

C. Trypsinization of cells. An 80% confluent monolayer of cos cells (1×10^7) was treated with 0.05% trypsin/0.02% versene solution (Biofluids) for 3-5 min at 37°C. Following detachment the trypsin was inactivated by the addition of an equal volume of media containing 10% fetal calf serum. The cells were then further diluted to a final concentration of 1×10^4 /ml. One ml of cells was plated in a 12 well dish and incubated with virus at a multiplicity of infection (MOI) of 260 for 1-2 hrs. Following attachment of the cells the media containing the virus was removed, the cells washed and fresh media was added. Control cells were plated at the same time but were not transduced until the next day. Transduction conditions were done as described above for the trypsinized cell group. The number of transduced cells was determined by staining 48-60 hrs post transduction and counted as described above.

Previous research had shown that binding and infection of AAV2 is inhibited by trypsin treatment of cells (26). Transduction of cos cells with rAAV2lacZ gene was also inhibited by trypsin treatment prior to transduction (Fig 4). In contrast trypsin treatment had a minimal effect on rAAV4lacZ transduction. This result and
5 the previous competition experiment are both consistent with the utilization of distinct cellular receptors for AAV2 and AAV4.

AAV4 is a distinct virus based on sequence analysis, physical properties of the virion, hemagglutination activity, and tissue tropism. The sequence data
10 indicates that AAV4 is a distinct virus from that of AAV2. In contrast to original reports, AAV4 contains two open reading frames which code for either Rep proteins or Capsid proteins. AAV4 contains additional sequence upstream of the p5 promoter which may affect promoter activity, packaging or particle stability. Furthermore, AAV4 contains an expanded Rep binding site in its ITR which could
15 alter its activity as an origin of replication or promoter. The majority of the differences in the Capsid proteins lies in regions which have been proposed to be on the exterior surface of the parvovirus. These changes are most likely responsible for the lack of cross-reacting antibodies, hemagglutinate activity, and the altered
tissue tropism compared to AAV2. Furthermore, in contrast to previous reports
20 AAV4 is able to transduce human as well as monkey cells.

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to
25 which this invention pertains.

Although the present process has been described with reference to specific details of certain embodiments thereof, it is not intended that such details should be regarded as limitations upon the scope of the invention except as and to the extent
30 that they are included in the accompanying claims.

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- 20

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Chiorini, John A.
Kotin, Robert M.
Safer, Brian

(ii) TITLE OF INVENTION: AAV4 VECTOR AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 22

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Needle & Rosenberg
(B) STREET: 127 Peachtree
(C) CITY: Atlanta
(D) STATE: Georgia
(E) COUNTRY: USA
(F) ZIP: 30303

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Selby, Elizabeth
(B) REGISTRATION NUMBER: 38,298
(C) REFERENCE/DOCKET NUMBER: 14014.0252

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4767 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) OTHER INFO: AAV4 genome

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTGGCCACTC CCTCTATGCG CGCTCGCTCA CTCACTCGGC CCTGGAGACC AAAGGTCTCC	60
AGACTGCCGG CCTCTGGCCG GCAGGGCCGA GTGAGTGAGC GAGCGCGCAT AGAGGGAGTG	120
GCCAACTCCA TCATCTAGGT TTGCCCCACTG ACGTCAATGT GACGTCCTAG GGTTAGGGAG	180
GTCCCTGTAT TAGCAGTCAC GTGAGTGTGCG TATTTGCGCG AGCGTAGCGG AGCGCATACC	240
AAGCTGCCAC GTCACAGCCA CGTGGTCCGT TTGCGACAGT TTGCGACACC ATGTGGTCAG	300
GAGGGTATAT AACCGCGAGT GAGCCAGCGA GGAGCTCCAT TTTGCCCCGCG AATTTTGAAC	360

GAGCAGCAGC	CATGCCGGGG	TTCTACGAGA	TCGTGCTGAA	GGTGCCCAGC	GACCTGGACG	420
AGCACCTGCC	CGGCATTTCT	GACTCTTTTG	TGAGCTGGGT	GGCCGAGAAG	GAATGGGAGC	480
TGCCGCCGGA	TTCTGACATG	GACTTGAATC	TGATTGAGCA	GGCACCCCTG	ACCGTGGCCG	540
AAAAGCTGCA	ACGCGAGTTC	CTGGTCGAGT	GGCGCCGCGT	GAGTAAGGCC	CCGGAGGCCC	600
TCTTCTTTGT	CCAGTTCGAG	AAGGGGGACA	GCTACTTCCA	CCTGCACATC	CTGGTGGAGA	660
CCGTGGGCGT	CAAATCCATG	GTGGTGGGCC	GCTACGTGAG	CCAGATTAAA	GAGAAGCTGG	720
TGACCCGCAT	CTACCGCGGG	GTCGAGCCGC	AGCTTCCGAA	CTGGTTCGCG	GTGACCAAGA	780
CGCGTAATGG	CGCCGGAGGC	GGGAACAAGG	TGGTGGACGA	CTGCTACATC	CCCAACTACC	840
TGCTCCCCAA	GACCCAGCCC	GAGCTCCAGT	GGGCGTGGAC	TAACATGGAC	CAGTATATAA	900
GCGCCTGTTT	GAATCTCGCG	GAGCGTAAAC	GGCTGGTGGC	GCAGCATCTG	ACGCACGTGT	960
CGCAGACGCA	GGAGCAGAAC	AAGGAAAACC	AGAACCCCCA	TTCTGACGCG	CCGGTCATCA	1020
GGTCAAAAAC	CTCCGCCAGG	TACATGGAGC	TGGTCGGGTG	GCTGGTGGAC	CGCGGGATCA	1080
CGTCAGAAAA	GCAATGGATC	CAGGAGGACC	AGGCGTCCTA	CATCTCCTTC	AACGCCGCCT	1140
CCAACTCGCG	GTCACAAATC	AAGGCCGCGC	TGGACAATGC	CTCCAAAATC	ATGAGCCTGA	1200
CAAAGACGGC	TCCGGACTAC	CTGGTGGGCC	AGAACCCGCC	GGAGGACATT	TCCAGCAACC	1260
GCATCTACCG	AATCCTCGAG	ATGAACGGGT	ACGATCCGCA	GTACGCGGCC	TCCGTCTTCC	1320
TGGGCTGGGC	GCAAAAGAAG	TTCGGGAAGA	GGAACACCAT	CTGGCTCTTT	GGGCCGGCCA	1380
CGACGGGTAA	AACCAACATC	GCGGAAGCCA	TCGCCCACGC	CGTGCCCTTC	TACGGCTGCG	1440
TGAACTGGAC	CAATGAGAAC	TTTCCGTTCA	ACGATTGCGT	CGACAAGATG	GTGATCTGGT	1500
GGGAGGAGGG	CAAGATGACG	GCCAAGGTCG	TAGAGAGCGC	CAAGGCCATC	CTGGGCGGAA	1560
GCAAGGTGCG	CGTGGACCAA	AAGTGCAAGT	CATCGGCCCA	GATCGACCCA	ACTCCCGTGA	1620
TCGTACCTC	CAACACCAAC	ATGTGCGCGG	TCATCGACGG	AAACTCGACC	ACCTTCGAGC	1680
ACCAACAACC	ACTCCAGGAC	CGGATGTTCA	AGTTCGAGCT	CACCAAGCGC	CTGGAGCACG	1740
ACTTTGGCAA	GGTCACCAAG	CAGGAAGTCA	AAGACTTTTT	CCGGTGGGCG	TCAGATCACG	1800
TGACCGAGGT	GACTCACGAG	TTTTACGTCA	GAAAGGGTGG	AGCTAGAAAG	AGGCCCCGCC	1860
CCAATGACGC	AGATATAAGT	GAGCCCAAGC	GGGCCTGTCC	GTCAGTTGCG	CAGCCATCGA	1920
CGTCAGACGC	GGAAGCTCCG	GTGGACTACG	CGGACAGGTA	CCAAAACAAA	TGTTCTCGTC	1980
ACGTGGGTAT	GAATCTGATG	CTTTTTCCCT	GCCGGCAATG	CGAGAGAATG	AATCAGAATG	2040
TGGACATTTG	CTTCACGCAC	GGGGTCATGG	ACTGTGCCGA	GTGCTTCCCC	GTGTCAGAAT	2100
CTCAACCCGT	GTCTGTCGTC	AGAAAGCGGA	CGTATCAGAA	ACTGTGTCCG	ATTCATCACA	2160
TCATGGGGAG	GGCGCCCGAG	GTGGCCTGCT	CGGCCTGCGA	ACTGGCCAAT	GTGGACTTGG	2220
ATGACTGTGA	CATGGAACAA	TAAATGACTC	AAACCAGATA	TGACTGACGG	TTACCTTCCA	2280

GATTGGCTAG	AGGACAACCT	CTCTGAAGGC	GTTTCGAGAGT	GGTGGGCGCT	GCAACCTGGA	2340
GGCCCTAAAC	CCAAGGCAAA	TCAACAACAT	CAGGACAACG	CTCGGGGTCT	TGTGCTTCCG	2400
GGTTACAAAT	ACCTCGGACC	CGGCAACGGA	CTCGACAAGG	GGGAACCCGT	CAACGCAGCG	2460
GACGCGGCAG	CCCTCGAGCA	CGACAAGGCC	TACGACCAGC	AGCTCAAGGC	CGGTGACAAC	2520
CCCTACCTCA	AGTACAACCA	CGCCGACGCG	GAGTTCCAGC	AGCGGCTTCA	GGGCGACACA	2580
CCGTTTGGGG	GCAACCTCGG	CAGAGCAGTC	TTCCAGGCCA	AAAAGAGGGT	TCTTGAACCT	2640
CTTGGTCTGG	TTGAGCAAGC	GGGTGAGACG	GCTCCTGGAA	AGAAGAGACC	GTTGATTGAA	2700
TCCCCCAGC	AGCCCGACTC	CTCCACGGGT	ATCGGCAAAA	AAGGCAAGCA	GCCGGCTAAA	2760
AAGAAGCTCG	TTTTCGAAGA	CGAAACTGGA	GCAGGCGACG	GACCCCTGA	GGGATCAACT	2820
TCCGGAGCCA	TGTCTGATGA	CAGTGAGATG	CGTGCAGCAG	CTGGCGGAGC	TGCAGTCGAG	2880
GGSGGACAAG	GTGCCGATGG	AGTGGGTAAT	GCCTCGGGTG	ATTGGCATTG	CGATTCCACC	2940
TGGTCTGAGG	GCCACGTCAC	GACCACCAGC	ACCAGAACCT	GGGTCTTGCC	CACCTACAAC	3000
AACCACCTNT	ACAAGCGACT	CGGAGAGAGC	CTGCAGTCCA	ACACCTACAA	CGGATTCTCC	3060
ACCCCTGGG	GATACTTTGA	CTTCAACCGC	TTCCACTGCC	ACTTCTCACC	ACGTGACTGG	3120
CAGCGACTCA	TCAACAACAA	CTGGGGCATG	CGACCCAAAG	CCATGCGGGT	CAAAATCTTC	3180
AACATCCAGG	TCAAGGAGGT	CACGACGTCG	AACGGCGAGA	CAACGGTGGC	TAATAACCTT	3240
ACCAGCACGG	TTCAGATCTT	TGCGGACTCG	TCGTACGAAC	TGCCGTACGT	GATGGATGCG	3300
GGTCAAGAGG	GCAGCCTGCC	TCCTTTTCCC	AACGACGTCT	TTATGGTGEE	CCAGTACGGC	3360
TACTGTGGAC	TGGTGACCGG	CAACACTTCG	CAGCAACAGA	CTGACAGAAA	TGCCTTCTAC	3420
TGCCTGGAGT	ACTTTCCTTC	GCAGATGCTG	CGGACTGGCA	ACAACTTTGA	AATTACGTAC	3480
AGTTTTGAGA	AGGTGCCTTT	CCACTCGATG	TACGCGCACA	GCCAGAGCCT	GGACCGGCTG	3540
ATGAACCTC	TCATCGACCA	GTACCTGTGG	GGACTGCAAT	CGACCACCAC	CGGAACCACC	3600
CTGAATGCCG	GGAAGTCCAC	CACCAACTTT	ACCAAGCTGC	GGCCTACCAA	CTTTTCCAAC	3660
TTTAAAAAGA	ACTGGCTGCC	CGGGCCTTCA	ATCAAGCAGC	AGGGCTTCTC	AAAGACTGCC	3720
AATCAAAACT	ACAAGATCCC	TGCCACCGGG	TCAGACAGTC	TCATCAAATA	CGAGACGCAC	3780
AGCACTCTGG	ACGGAAGATG	GAGTGCCCTG	ACCCCGGAC	CTCCAATGGC	CACGGCTGGA	3840
CCTGCGGACA	GCAAGTTCAG	CAACAGCCAG	CTCATCTTTG	CGGGGCCTAA	ACAGAACGGC	3900
AACACGGCCA	CCGTACCCGG	GACTCTGATC	TTACCTCTG	AGGAGGAGCT	GGCAGCCACC	3960
AACGCCACCG	ATACGGACAT	GTGGGGCAAC	CTACCTGGCG	GTGACCAGAG	CAACAGCAAC	4020
CTGCCGACCG	TGGACAGACT	GACAGCCTTG	GGAGCCGTGC	CTGGAATGGT	CTGGCAAAAC	4080
AGAGACATTT	ACTACCAGGG	TCCCATTTGG	GCCAAGATTC	CTCATACCGA	TGGACACTTT	4140
CACCCCTCAC	CGCTGATTGG	TGGGTTTGGG	CTGAAACACC	CGCCTCCTCA	AATTTTTATC	4200

AAGAACACCC CGGTACCTGC GAATCCTGCA ACGACCTTCA GCTCTACTCC GGTAAACTCC 4260
 TTCATTACTC AGTACAGCAC TGGCCAGGTG TCGGTGCAGA TTGACTGGGA GATCCAGAAG 4320
 GAGCGGTCCA AACGCTGGAA CCCCAGAGTC CAGTTTACCT CCAACTACGG ACAGCAAAAC 4380
 TCTCTGTTGT GGGCTCCCGA TCGGCTGGG AAATACACTG AGCCTAGGGC TATCGGTACC 4440
 CGCTACCTCA CCCACCACCT GTAATAACCT GTTAATCAAT AAACCGGTTT ATTCGTTTCA 4500
 GTTGAAC TTT GGTCTCCGTG TCCTTCTTAT CTTATCTCGT TTCCATGGCT ACTGCGTACA 4560
 TAAGCAGCGG CCTGCGGCGC TTGCGCTTCG CGGTTTACAA CTGCCGGTTA ATCAGTAACT 4620
 TCTGGCAAAC CAGATGATGG AGTTGGCCAC ATTAGCTATG CGCGCTCGCT CACTCACTCG 4680
 GCCCTGGAGA CCAAAGGTCT CCAGACTGCC GGCCTCTGGC CGGCAGGGCC GAGTGAGTGA 4740
 GCGAGCGCGC ATAGAGGGAG TGGCCAA 4767

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 624 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) OTHER INFO: AAV4 Rep protein (full length)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Pro Gly Phe Tyr Glu Ile Val Leu Lys Val Pro Ser Asp Leu Asp
 1 5 10 15
 Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Ser Trp Val Ala Glu
 20 25 30
 Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
 35 40 45
 Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Glu Phe Leu
 50 55 60
 Val Glu Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
 65 70 75 80
 Gln Phe Glu Lys Gly Asp Ser Tyr Phe His Leu His Ile Leu Val Glu
 85 90 95
 Thr Val Gly Val Lys Ser Met Val Val Gly Arg Tyr Val Ser Gln Ile
 100 105 110
 Lys Glu Lys Leu Val Thr Arg Ile Tyr Arg Gly Val Glu Pro Gln Leu
 115 120 125
 Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
 130 135 140
 Asn Lys Val Val Asp Asp Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
 145 150 155 160

50

Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Asp Gln Tyr Ile
 165 170 175
 Ser Ala Cys Leu Asn Leu Ala Glu Arg Lys Arg Leu Val Ala Gln His
 180 185 190
 Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Gln Asn
 195 200 205
 Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
 210 215 220
 Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
 225 230 235 240
 Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
 245 250 255
 Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Ser Lys
 260 265 270
 Ile Met Ser Leu Thr Lys Thr Ala Pro Asp Tyr Leu Val Gly Gln Asn
 275 280 285
 Pro Pro Glu Asp Ile Ser Ser Asn Arg Ile Tyr Arg Ile Leu Glu Met
 290 295 300
 Asn Gly Tyr Asp Pro Gln Tyr Ala Ala Ser Val Phe Leu Gly Trp Ala
 305 310 315 320
 Gln Lys Lys Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
 325 330 335
 Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
 340 345 350

Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
 355 360 365
 Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
 370 375 380
 Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
 385 390 395 400
 Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
 405 410 415
 Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
 420 425 430
 Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
 435 440 445
 Glu Leu Thr Lys Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
 450 455 460
 Glu Val Lys Asp Phe Phe Arg Trp Ala Ser Asp His Val Thr Glu Val
 465 470 475 480
 Thr His Glu Phe Tyr Val Arg Lys Gly Gly Ala Arg Lys Arg Pro Ala
 485 490 495

Pro Asn Asp Ala Asp Ile Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
500 505 510

Ala Gln Pro Ser Thr Ser Asp Ala Glu Ala Pro Val Asp Tyr Ala Asp
515 520 525

Arg Tyr Gln Asn Lys Cys Ser Arg His Val Gly Met Asn Leu Met Leu
530 535 540

Phe Pro Cys Arg Gln Cys Glu Arg Met Asn Gln Asn Val Asp Ile Cys
545 550 555 560

Phe Thr His Gly Val Met Asp Cys Ala Glu Cys Phe Pro Val Ser Glu
565 570 575

Ser Gln Pro Val Ser Val Val Arg Lys Arg Thr Tyr Gln Lys Leu Cys
580 585 590

Pro Ile His His Ile Met Gly Arg Ala Pro Glu Val Ala Cys Ser Ala
595 600 605

Cys Glu Leu Ala Asn Val Asp Leu Asp Asp Cys Asp Met Glu Gln *

610 615 620

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1872 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) OTHER INFO: AAV4 Rep gene (full length)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1872

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG CCG GGG TTC TAC GAG ATC GTG CTG AAG GTG CCC AGC GAC CTG GAC	48
Met Pro Gly Phe Tyr Glu Ile Val Leu Lys Val Pro Ser Asp Leu Asp	
1 5 10 15	
GAG CAC CTG CCC GGC ATT TCT GAC TCT TTT GTG AGC TGG GTG GCC GAG	96
Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Ser Trp Val Ala Glu	
20 25 30	
AAG GAA TGG GAG CTG CCG CCG GAT TCT GAC ATG GAC TTG AAT CTG ATT	144
Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile	
35 40 45	
GAG CAG GCA CCC CTG ACC GTG GCC GAA AAG CTG CAA CGC GAG TTC CTG	192
Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Glu Phe Leu	
50 55 60	

GTC GAG TGG CGC CGC GTG AGT AAG GCC CCG GAG GCC CTC TTC TTT GTC Val Glu Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val 65 70 75 80	240
CAG TTC GAG AAG GGG GAC AGC TAC TTC CAC CTG CAC ATC CTG GTG GAG Gln Phe Glu Lys Gly Asp Ser Tyr Phe His Leu His Ile Leu Val Glu 85 90 95	288
ACC GTG GGC GTC AAA TCC ATG GTG GTG GGC CGC TAC GTG AGC CAG ATT Thr Val Gly Val Lys Ser Met Val Val Gly Arg Tyr Val Ser Gln Ile 100 105 110	336
AAA GAG AAG CTG GTG ACC CGC ATC TAC CGC GGG GTC GAG CCG CAG CTT Lys Glu Lys Leu Val Thr Arg Ile Tyr Arg Gly Val Glu Pro Gln Leu 115 120 125	384
CCG AAC TGG TTC GCG GTG ACC AAG ACG CGT AAT GGC GCC GGA GGC GGG Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly 130 135 140	432
AAC AAG GTG GTG GAC GAC TGC TAC ATC CCC AAC TAC CTG CTC CCC AAG Asn Lys Val Val Asp Asp Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys 145 150 155 160	480
ACC CAG CCC GAG CTC CAG TGG GCG TGG ACT AAC ATG GAC CAG TAT ATA Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Asp Gln Tyr Ile 165 170 175	528
AGC GCC TGT TTG AAT CTC GCG GAG CGT AAA CGG CTG GTG GCG CAG CAT Ser Ala Cys Leu Asn Leu Ala Glu Arg Lys Arg Leu Val Ala Gln His 180 185 190	576
CTG ACG CAC GTG TCG CAG ACG CAG GAG CAG AAC AAG GAA AAC CAG AAC Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Gln Asn 195 200 205	624
CCC AAT TCT GAC GCG CCG GTC ATC AGG TCA AAA ACC TCC GCC AGG TAC Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr 210 215 220	672
ATG GAG CTG GTC GGG TGG CTG GTG GAC CGC GGG ATC ACG TCA GAA AAG Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys 225 230 235 240	720
CAA TGG ATC CAG GAG GAC CAG GCG TCC TAC ATC TCC TTC AAC GCC GCC Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala 245 250 255	768
TCC AAC TCG CGG TCA CAA ATC AAG GCC GCG CTG GAC AAT GCC TCC AAA Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Ser Lys 260 265 270	816
ATC ATG AGC CTG ACA AAG ACG GCT CCG GAC TAC CTG GTG GGC CAG AAC Ile Met Ser Leu Thr Lys Thr Ala Pro Asp Tyr Leu Val Gly Gln Asn 275 280 285	864
CCG CCG GAG GAC ATT TCC AGC AAC CGC ATC TAC CGA ATC CTC GAG ATG Pro Pro Glu Asp Ile Ser Ser Asn Arg Ile Tyr Arg Ile Leu Glu Met 290 295 300	912
AAC GGG TAC GAT CCG CAG TAC GCG GCC TCC GTC TTC CTG GGC TGG GCG Asn Gly Tyr Asp Pro Gln Tyr Ala Ala Ser Val Phe Leu Gly Trp Ala 305 310 315 320	960

CAA AAG AAG TTC GGG AAG AGG AAC ACC ATC TGG CTC TTT GGG CCG GCC	1008
Gln Lys Lys Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala	
325 330 335	
ACG ACG GGT AAA ACC AAC ATC GCG GAA GCC ATC GCC CAC GCC GTG CCC	1056
Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro	
340 345 350	
TTC TAC GGC TGC GTG AAC TGG ACC AAT GAG AAC TTT CCG TTC AAC GAT	1104
Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp	
355 360 365	
TGC GTC GAC AAG ATG GTG ATC TGG TGG GAG GAG GGC AAG ATG ACG GCC	1152
Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala	
370 375 380	
AAG GTC GTA GAG AGC GCC AAG GCC ATC CTG GGC GGA AGC AAG GTG CGC	1200
Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg	
385 390 395 400	
GTG GAC CAA AAG TGC AAG TCA TCG GCC CAG ATC GAC CCA ACT CCC GTG	1248
Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val	
405 410 415	
ATC GTC ACC TCC AAC ACC AAC ATG TGC GCG GTC ATC GAC GGA AAC TCG	1296
Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser	
420 425 430	
ACC ACC TTC GAG CAC CAA CAA CCA CTC CAG GAC CGG ATG TTC AAG TTC	1344
Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe	
435 440 445	
GAG CTC ACC AAG CGC CTG GAG CAC GAC TTT GGC AAG GTC ACC AAG CAG	1392
Glu Leu Thr Lys Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln	
450 455 460	
GAA GTC AAA GAC TTT TTC CGG TGG GCG TCA GAT CAC GTG ACC GAG GTG	1440
Glu Val Lys Asp Phe Phe Arg Trp Ala Ser Asp His Val Thr Glu Val	
465 470 475 480	
ACT CAC GAG TTT TAC GTC AGA AAG GGT GGA GCT AGA AAG AGG CCC GCC	1488
Thr His Glu Phe Tyr Val Arg Lys Gly Gly Ala Arg Lys Arg Pro Ala	
485 490 495	
CCC AAT GAC GCA GAT ATA AGT GAG CCC AAG CGG GCC TGT CCG TCA GTT	1536
Pro Asn Asp Ala Asp Ile Ser Glu Pro Lys Arg Ala Cys Pro Ser Val	
500 505 510	
GCG CAG CCA TCG ACG TCA GAC GCG GAA GCT CCG GTG GAC TAC GCG GAC	1584
Ala Gln Pro Ser Thr Ser Asp Ala Glu Ala Pro Val Asp Tyr Ala Asp	
515 520 525	
AGG TAC CAA AAC AAA TGT TCT CGT CAC GTG GGT ATG AAT CTG ATG CTT	1632
Arg Tyr Gln Asn Lys Cys Ser Arg His Val Gly Met Asn Leu Met Leu	
530 535 540	
TTT CCC TGC CGG CAA TGC GAG AGA ATG AAT CAG AAT GTG GAC ATT TGC	1680
Phe Pro Cys Arg Gln Cys Glu Arg Met Asn Gln Asn Val Asp Ile Cys	
545 550 555 560	
TTC ACG CAC GGG GTC ATG GAC TGT GCC GAG TGC TTC CCC GTG TCA GAA	1728
Phe Thr His Gly Val Met Asp Cys Ala Glu Cys Phe Pro Val Ser Glu	
565 570 575	

54

TCT CAA CCC GTG TCT GTC GTC AGA AAG CGG ACG TAT CAG AAA CTG TGT	1776
Ser Gln Pro Val Ser Val Val Arg Lys Arg Thr Tyr Gln Lys Leu Cys	
580 585 590	
CCG ATT CAT CAC ATC ATG GGG AGG GCG CCC GAG GTG GCC TGC TCG GCC	1824
Pro Ile His His Ile Met Gly Arg Ala Pro Glu Val Ala Cys Ser Ala	
595 600 605	
TGC GAA CTG GCC AAT GTG GAC TTG GAT GAC TGT GAC ATG GAA CAA TAA	1872
Cys Glu Leu Ala Asn Val Asp Leu Asp Asp Cys Asp Met Glu Gln *	
610 615 620	

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 734 amino acids

(B) TYPE: amino acid

(ii) MOLECULE TYPE:

(A) DESCRIPTION: protein

(ix) OTHER INFO: AAV4 capsid protein VP1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Thr Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser Glu	
1 5 10 15	
Gly Val Arg Glu Trp Trp Ala Leu Gln Pro Gly Ala Pro Lys Pro Lys	
20 25 30	
Ala Asn Gln Gln His Gln Asp Asn Ala Arg Gly Leu Val Leu Pro Gly	
35 40 45	
Tyr Lys Tyr Leu Gly Pro Gly Asn Gly Leu Asp Lys Gly Glu Pro Val	
50 55 60	
Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp Gln	
65 70 75 80	
Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala Asp	
85 90 95	
Ala Glu Phe Gln Gln Arg Leu Gln Gly Asp Thr Ser Phe Gly Gly Asn	
100 105 110	
Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro Leu	
115 120 125	
Gly Leu Val Glu Gln Ala Gly Glu Thr Ala Pro Gly Lys Lys Arg Pro	
130 135 140	
Leu Ile Glu Ser Pro Gln Gln Pro Asp Ser Ser Thr Gly Ile Gly Lys	
145 150 155 160	
Lys Gly Lys Gln Pro Ala Lys Lys Lys Leu Val Phe Glu Asp Glu Thr	
165 170 175	
Gly Ala Gly Asp Gly Pro Pro Glu Gly Ser Thr Ser Gly Ala Met Ser	
180 185 190	
Asp Asp Ser Glu Met Arg Ala Ala Ala Gly Gly Ala Ala Val Glu Gly	
195 200 205	
Gly Gln Gly Ala Asp Gly Val Gly Asn Ala Ser Gly Asp Trp His Cys	
210 215 220	
Asp Ser Thr Trp Ser Glu Gly His Val Thr Thr Thr Ser Thr Arg Thr	
225 230 235 240	
Trp Val Leu Pro Thr Tyr Asn Asn His Leu Tyr Lys Arg Leu Gly Glu	
245 250 255	
Ser Leu Gln Ser Asn Thr Tyr Asn Gly Phe Ser Thr Pro Trp Gly Tyr	
260 265 270	

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2208 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ix) OTHER INFO: AAV4 capsid protein VP1 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGACTGACG	GTTACCTTCC	AGATTGGCTA	GAGGACAACC	TCTCTGAAGG	CGTTCGAGAG	60
TGGTGGGCGC	TGCAACCTGG	AGCCCCTAAA	CCCAAGGCAA	ATCAACAACA	TCAGGACAAC	120
GCTCGGGGTC	TTGTGCTTCC	GGGTTACAAA	TACCTCGGAC	CCGGCAACGG	ACTCGACAAG	180
GGGGAACCCG	TCAACGCAGC	GGACGCGGCA	GCCCTCGAGC	ACGACAAGGC	CTACGACCAG	240
CAGCTCAAGG	CCGGTGACAA	CCCCTACCTC	AAGTACAACC	ACGCCGACGC	GGAGTTCCAG	300
CAGCGGCTTC	AGGGCGACAC	ATCGTTTGGG	GGCAACCTCG	GCAGAGCAGT	CTTCCAGGCC	360
AAAAAGAGGG	TTCTTGAACC	TCTTGGTCTG	GTTGAGCAAG	CGGGTGAGAC	GGCTCCTGGA	420
AAGAAGAGAC	CGTTGATTGA	ATCCCCCAG	CAGCCCGACT	CCTCCACGGG	TATCGGCAAA	480
AAAGGCAAGC	AGCCGGCTAA	AAAGAAGCTC	GTTTTCTGAAG	ACGAAACTGG	AGCAGGCGAC	540
GGACCCCTTG	AGGGATCAAC	TTCCGGAGCC	ATGTCTGATG	ACAGTGAGAT	GCGTGCAGCA	600
GCTGGCGGAG	CTGCAGTCGA	GGGSGGACAA	GGTGCCGATG	GAGTGGGTAA	TGCCTCGGGT	660
GATTGGCATT	GCGATTCCAC	CTGGTCTGAG	GGCCACGTCA	CGACCACCAG	CACCAGAACC	720
TGGGTCTTGC	CCACCTACAA	CAACCACCTN	TACAAGCGAC	TCGGAGAGAG	CCTCGAGTCC	780
AACACCTACA	ACGGATTCTC	CACCCCTTGG	GGATACTTTG	ACTTCAACCG	CTTCCAGTGC	840
CACTTCTCAC	CACGTGACTG	GCAGCGACTC	ATCAACAACA	ACTGGGGCAT	GCGACCCAAA	900
GCCATGCGGG	TCAAAATCTT	CAACATCCAG	GTCAAGGAGG	TCACGACGTC	GAACGGCGAG	960
ACAACGGTGG	CTAATAACCT	TACCAGCACG	GTTTCAGATCT	TTGCGGACTC	GTCTGACGAA	1020
CTGCCGTACG	TGATGGATGC	GGGTCAAGAG	GGCAGCCTGC	CTCCTTTTCC	CAACGACGTC	1080
TTTATGGTGC	CCCAGTACGG	CTACTGTGGA	CTGGTGACCG	GCAACACTTC	GCAGCAACAG	1140
ACTGACAGAA	ATGCCTTCTA	CTGCCTGGAG	TACTTTCCTT	CGCAGATGCT	GCGGACTGGC	1200
AACAACCTTG	AAATTACGTA	CAGTTTTGAG	AAGGTGCCTT	TCCACTCGAT	GTACGCGCAC	1260
AGCCAGAGCC	TGGACCGGCT	GATGAACCTT	CTCATCGACC	AGTACCTGTG	GGGACTGCAA	1320
TCGACCACCA	CCGGAACCAC	CCTGAATGCC	GGGACTGCCA	CCACCAACTT	TACCAAGCTG	1380
CGGCCTACCA	ACTTTTCCAA	CTTTAAAAAG	AAGTGGCTGC	CCGGGCCTTC	AATCAAGCAG	1440
CAGGGCTTCT	CAAAGACTGC	CAATCAAAAC	TACAAGATCC	CTGCCACCGG	GTCAGACAGT	1500
CTCATCAAAT	ACGAGACGCA	CAGCACTCTG	GACGGAAGAT	GGAGTGCCCT	GACCCCGGGA	1560
CCTCCAATGG	CCACGGCTGG	ACCTGCGGAG	AGCAAGTTCA	GCAACAGCCA	GCTCATCTTT	1620
GCGGGGCCCTA	AACAGAACGG	CAACACGGCC	ACCGTACCCG	GGACTCTGAT	CTTCACCTCT	1680
GAGGAGGAGC	TGGCAGCCAC	CAACGCCACC	GATACGGACA	TGTGGGGCAA	CCTACCTGGC	1740
GGTGACCAGA	GCAACAGCAA	CCTGCCGACC	GTGGACAGAC	TGACAGCCTT	GGGAGCCGTG	1800
CCTGGAATGG	TCTGGCAAAA	CAGAGACATT	TACTACCAGG	GTCCCATTG	GGCCAAGATT	1860
CCTCATACCG	ATGGACACTT	TCACCCCTCA	CCGCTGATTG	GTGGGTTTGG	GCTGAAACAC	1920
CCGCCTCCTC	AAATTTTAT	CAAGAACACC	CCGGTACCTG	CGAATCCTGC	AACGACCTTC	1980
AGCTCTACTC	CGGTAAACTC	CTTCATTACT	CAGTACAGCA	CTGGCCAGGT	GTCGGTGCAG	2040
ATTGACTGGG	AGATCCAGAA	GGAGCGGTCC	AAACGCTGGA	ACCCCGAGGT	CCAGTTTACC	2100
TCCAACACG	GACAGCAAAA	CTCTCTGTTG	TGGGCTCCCG	ATGCGGCTGG	GAAATACACT	2160
GAGCCTAGGG	CTATCGGTAC	CCGCTACCTC	ACCCACCACC	TGTAATAA		2208

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 125 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ix) OTHER INFO: AAV4 ITR "flip" orientation

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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TTGGCCACTC CCTCTATGCG CGCTCGCTCA CTCACTCGGC CCTGGAGACC AAAGGTCTCC 60
 AGACTGCCGG CCTCTGGCCG GCAGGGCCGA GTGAGTGAGC GAGCGCGCAT AGAGGGAGTG 120
 GCCAA 125

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 245 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ix) OTHER INFO: AAV4 p5 promoter

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CTCCATCATC TAGGTTTGCC CACTGACGTC AATGTGACGT CCTAGGGTTA GGGAGGTCCC 60
 TGTATTAGCA GTCACGTGAG TGTCGTATTT CGCGGAGCGT AGCGGAGCGC ATACCAAGCT 120
 GCCACGTCAC AGCCACGTGG TCCGTTTGCG ACAGTTTGCG ACACCATGTG GTCAGGAGGG 180
 TATATAACCG CGAGTGAGCC AGCGAGGAGC TCCATTTTGC CCGCGAATT TGAACGAGCA 240
 GCAGC 245

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 313 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE:

(A) DESCRIPTION: protein

(ix) OTHER INFO: AAV4 Rep protein 40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met	Glu	Leu	Val	Gly	Trp	Leu	Val	Asp	Arg	Gly	Ile	Thr	Ser	Glu	Lys	1	5	10	15
Gln	Trp	Ile	Gln	Glu	Asp	Gln	Ala	Ser	Tyr	Ile	Ser	Phe	Asn	Ala	Ala	20	25	30	
Ser	Asn	Ser	Arg	Ser	Gln	Ile	Lys	Ala	Ala	Leu	Asp	Asn	Ala	Ser	Lys	35	40	45	
Ile	Met	Ser	Leu	Thr	Lys	Thr	Ala	Pro	Asp	Tyr	Leu	Val	Gly	Gln	Asn	50	55	60	
Pro	Pro	Glu	Asp	Ile	Ser	Ser	Asn	Arg	Ile	Tyr	Arg	Ile	Leu	Glu	Met	65	70	75	80
Asn	Gly	Tyr	Asp	Pro	Gln	Tyr	Ala	Ala	Ser	Val	Phe	Leu	Gly	Trp	Ala	85	90	95	
Gln	Lys	Lys	Phe	Gly	Lys	Arg	Asn	Thr	Ile	Trp	Leu	Phe	Gly	Pro	Ala	100	105	110	
Thr	Thr	Gly	Lys	Thr	Asn	Ile	Ala	Glu	Ala	Ile	Ala	His	Ala	Val	Pro	115	120	125	
Phe	Tyr	Gly	Cys	Val	Asn	Trp	Thr	Asn	Glu	Asn	Phe	Pro	Phe	Asn	Asp	130	135	140	
Cys	Val	Asp	Lys	Met	Val	Ile	Trp	Trp	Glu	Glu	Gly	Lys	Met	Thr	Ala	145	150	155	160
Lys	Val	Val	Glu	Ser	Ala	Lys	Ala	Ile	Leu	Gly	Gly	Ser	Lys	Val	Arg	165	170	175	

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Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
      180      185      190
Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
      195      200      205
Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
      210      215      220
Glu Leu Thr Lys Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
      225      230      235      240
Glu Val Lys Asp Phe Phe Arg Trp Ala Ser Asp His Val Thr Glu Val
      245      250      255
Thr His Glu Phe Tyr Val Arg Lys Gly Gly Ala Arg Lys Arg Pro Ala
      260      265      270
Pro Asn Asp Ala Asp Ile Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
      275      280      285
Ala Gln Pro Ser Thr Ser Asp Ala Glu Ala Pro Val Asp Tyr Ala Asp
      290      295      300
Arg Leu Ala Arg Gly Gln Pro Leu Xaa
      305      310

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(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE:

- (A) DESCRIPTION: protein

(ix) OTHER INFO: AAV4 Rep protein 52

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
  1      5      10      15
Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
      20      25      30
Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Ser Lys
      35      40      45
Ile Met Ser Leu Thr Lys Thr Ala Pro Asp Tyr Leu Val Gly Gln Asn
      50      55      60
Pro Pro Glu Asp Ile Ser Ser Asn Arg Ile Tyr Arg Ile Leu Glu Met
      65      70      75      80
Asn Gly Tyr Asp Pro Gln Tyr Ala Ala Ser Val Phe Leu Gly Trp Ala
      85      90      95
Gln Lys Lys Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
      100      105      110
Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
      115      120      125
Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
      130      135      140
Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
      145      150      155      160
Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
      165      170      175
Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
      180      185      190
Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
      195      200      205
Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
      210      215      220

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Glu Leu Thr Lys Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
 225 230 235 240
 Glu Val Lys Asp Phe Phe Arg Trp Ala Ser Asp His Val Thr Glu Val
 245 250 255
 Thr His Glu Phe Tyr Val Arg Lys Gly Gly Ala Arg Lys Arg Pro Ala
 260 265 270
 Pro Asn Asp Ala Asp Ile Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
 275 280 285
 Ala Gln Pro Ser Thr Ser Asp Ala Glu Ala Pro Val Asp Tyr Ala Asp
 290 295 300
 Arg Tyr Gln Asn Lys Cys Ser Arg His Val Gly Met Asn Leu Met Leu
 305 310 315 320
 Phe Pro Cys Arg Gln Cys Glu Arg Met Asn Gln Asn Val Asp Ile Cys
 325 330 335
 Phe Thr His Gly Val Met Asp Cys Ala Glu Cys Phe Pro Val Ser Glu
 340 345 350
 Ser Gln Pro Val Ser Val Val Arg Lys Arg Thr Tyr Gln Lys Leu Cys
 355 360 365
 Pro Ile His His Ile Met Gly Arg Ala Pro Glu Val Ala Cys Ser Ala
 370 375 380
 Cys Glu Leu Ala Asn Val Asp Leu Asp Asp Cys Asp Met Glu Gln
 385 390 395

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 537 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE:

- (A) DESCRIPTION: protein

(ix) OTHER INFO: AAV4 Rep protein 68

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Pro Gly Phe Tyr Glu Ile Val Leu Lys Val Pro Ser Asp Leu Asp
 1 5 10 15
 Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Ser Trp Val Ala Glu
 20 25 30
 Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
 35 40 45
 Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Glu Phe Leu
 50 55 60
 Val Glu Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
 65 70 75 80
 Gln Phe Glu Lys Gly Asp Ser Tyr Phe His Leu His Ile Leu Val Glu
 85 90 95
 Thr Val Gly Val Lys Ser Met Val Val Gly Arg Tyr Val Ser Gln Ile
 100 105 110
 Lys Glu Lys Leu Val Thr Arg Ile Tyr Arg Gly Val Glu Pro Gln Leu
 115 120 125
 Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
 130 135 140
 Asn Lys Val Val Asp Asp Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
 145 150 155 160
 Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Asp Gln Tyr Ile
 165 170 175
 Ser Ala Cys Leu Asn Leu Ala Glu Arg Lys Arg Leu Val Ala Gln His
 180 185 190

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Leu	Thr	His	Val	Ser	Gln	Thr	Gln	Glu	Gln	Asn	Lys	Glu	Asn	Gln	Asn
		195					200					205			
Pro	Asn	Ser	Asp	Ala	Pro	Val	Ile	Arg	Ser	Lys	Thr	Ser	Ala	Arg	Tyr
	210					215					220				
Met	Glu	Leu	Val	Gly	Trp	Leu	Val	Asp	Arg	Gly	Ile	Thr	Ser	Glu	Lys
225					230					235					240
Gln	Trp	Ile	Gln	Glu	Asp	Gln	Ala	Ser	Tyr	Ile	Ser	Phe	Asn	Ala	Ala
				245					250					255	
Ser	Asn	Ser	Arg	Ser	Gln	Ile	Lys	Ala	Ala	Leu	Asp	Asn	Ala	Ser	Lys
			260					265						270	
Ile	Met	Ser	Leu	Thr	Lys	Thr	Ala	Pro	Asp	Tyr	Leu	Val	Gly	Gln	Asn
		275					280						285		
Pro	Pro	Glu	Asp	Ile	Ser	Ser	Asn	Arg	Ile	Tyr	Arg	Ile	Leu	Glu	Met
	290					295					300				
Asn	Gly	Tyr	Asp	Pro	Gln	Tyr	Ala	Ala	Ser	Val	Phe	Leu	Gly	Trp	Ala
305					310					315					320
Gln	Lys	Lys	Phe	Gly	Lys	Arg	Asn	Thr	Ile	Trp	Leu	Phe	Gly	Pro	Ala
				325					330					335	
Thr	Thr	Gly	Lys	Thr	Asn	Ile	Ala	Glu	Ala	Ile	Ala	His	Ala	Val	Pro
			340					345						350	
Phe	Tyr	Gly	Cys	Val	Asn	Trp	Thr	Asn	Glu	Asn	Phe	Pro	Phe	Asn	Asp
		355					360					365			
Cys	Val	Asp	Lys	Met	Val	Ile	Trp	Trp	Glu	Glu	Gly	Lys	Met	Thr	Ala
	370					375					380				
Lys	Val	Val	Glu	Ser	Ala	Lys	Ala	Ile	Leu	Gly	Gly	Ser	Lys	Val	Arg
385					390					395					400
Val	Asp	Gln	Lys	Cys	Lys	Ser	Ser	Ala	Gln	Ile	Asp	Pro	Thr	Pro	Val
				405					410					415	
Ile	Val	Thr	Ser	Asn	Thr	Asn	Met	Cys	Ala	Val	Ile	Asp	Gly	Asn	Ser
			420					425					430		
Thr	Thr	Phe	Glu	His	Gln	Gln	Pro	Leu	Gln	Asp	Arg	Met	Phe	Lys	Phe
		435					440					445			
Glu	Leu	Thr	Lys	Arg	Leu	Glu	His	Asp	Phe	Gly	Lys	Val	Thr	Lys	Gln
	450					455					460				
Glu	Val	Lys	Asp	Phe	Phe	Arg	Trp	Ala	Ser	Asp	His	Val	Thr	Glu	Val
465					470					475					480
Thr	His	Glu	Phe	Tyr	Val	Arg	Lys	Gly	Gly	Ala	Arg	Lys	Arg	Pro	Ala
				485					490					495	
Pro	Asn	Asp	Ala	Asp	Ile	Ser	Glu	Pro	Lys	Arg	Ala	Cys	Pro	Ser	Val
			500					505					510		
Ala	Gln	Pro	Ser	Thr	Ser	Asp	Ala	Glu	Ala	Pro	Val	Asp	Tyr	Ala	Asp
		515					520					525			
Arg	Leu	Ala	Arg	Gly	Gln	Pro	Leu	Xaa							
	530					535									

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 623 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE:

- (A) DESCRIPTION: protein

(ix) OTHER INFO: AAV4 Rep protein 78

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Pro Gly Phe Tyr Glu Ile Val Leu Lys Val Pro Ser Asp Leu Asp
1 5 10 15

Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Ser Trp Val Ala Glu
 20 25 30
 Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
 35 40 45
 Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Glu Phe Leu
 50 55 60
 Val Glu Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
 65 70 75 80
 Gln Phe Glu Lys Gly Asp Ser Tyr Phe His Leu His Ile Leu Val Glu
 85 90 95
 Thr Val Gly Val Lys Ser Met Val Val Gly Arg Tyr Val Ser Gln Ile
 100 105 110
 Lys Glu Lys Leu Val Thr Arg Ile Tyr Arg Gly Val Glu Pro Gln Leu
 115 120 125
 Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
 130 135 140
 Asn Lys Val Val Asp Asp Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
 145 150 155 160
 Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Asp Gln Tyr Ile
 165 170 175
 Ser Ala Cys Leu Asn Leu Ala Glu Arg Lys Arg Leu Val Ala Gln His
 180 185 190
 Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Gln Asn
 195 200 205
 Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
 210 215 220
 Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
 225 230 235 240
 Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
 245 250 255
 Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Ser Lys
 260 265 270
 Ile Met Ser Leu Thr Lys Thr Ala Pro Asp Tyr Leu Val Gly Gln Asn
 275 280 285
 Pro Pro Glu Asp Ile Ser Ser Asn Arg Ile Tyr Arg Ile Leu Glu Met
 290 295 300
 Asn Gly Tyr Asp Pro Gln Tyr Ala Ala Ser Val Phe Leu Gly Trp Ala
 305 310 315 320
 Gln Lys Lys Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
 325 330 335
 Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
 340 345 350
 Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
 355 360 365
 Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
 370 375 380
 Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
 385 390 395 400
 Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
 405 410 415
 Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
 420 425 430
 Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
 435 440 445
 Glu Leu Thr Lys Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
 450 455 460
 Glu Val Lys Asp Phe Phe Arg Trp Ala Ser Asp His Val Thr Glu Val
 465 470 475 480
 Thr His Glu Phe Tyr Val Arg Lys Gly Gly Ala Arg Lys Arg Pro Ala
 485 490 495
 Pro Asn Asp Ala Asp Ile Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
 500 505 510

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Ala Gln Pro Ser Thr Ser Asp Ala Glu Ala Pro Val Asp Tyr Ala Asp
 515 520 525
 Arg Tyr Gln Asn Lys Cys Ser Arg His Val Gly Met Asn Leu Met Leu
 530 535 540
 Phe Pro Cys Arg Gln Cys Glu Arg Met Asn Gln Asn Val Asp Ile Cys
 545 550 555 560
 Phe Thr His Gly Val Met Asp Cys Ala Glu Cys Phe Pro Val Ser Glu
 565 570 575
 Ser Gln Pro Val Ser Val Val Arg Lys Arg Thr Tyr Gln Lys Leu Cys
 580 585 590
 Pro Ile His His Ile Met Gly Arg Ala Pro Glu Val Ala Cys Ser Ala
 595 600 605
 Cys Glu Leu Ala Asn Val Asp Leu Asp Asp Cys Asp Met Glu Gln
 610 615 620

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 939 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ix) OTHER INFO: AAV4 Rep 40 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ATGGAGCTGG	TCGGGTGGCT	GGTGGACCGC	GGGATCACGT	CAGAAAAGCA	ATGGATCCAG	60
GAGGACCAGG	CGTCCTACAT	CTCCTTCAAC	GCCGCCTCCA	ACTCGCGGTC	ACAAATCAAG	120
GCCGCGCTGG	ACAATGCCTC	CAAAATCATG	AGCCTGACAA	AGACGGCTCC	GGACTACCTG	180
GTGGGCCAGA	ACCCGCCGGA	GGACATTTCC	AGCAACCGCA	TCTACCGAAT	CCTCGAGATG	240
AACGGGTACG	ATCCGCAGTA	CGEGGGEETCC	GTCTTCCTGG	GCTGGGCGCA	AAAGAAGTTC	300
GGGAAGAGGA	ACACCATCTG	GCTCTTTGGG	CCGGCCACGA	CGGGTAAAC	CAACATCGCG	360
GAAGCCATCG	CCCACGCCGT	GCCCTTCTAC	GGCTGCGTGA	ACTGGACCAA	TGAGAACTTT	420
CCGTTCACG	ATTGCGTCGA	CAAGATGGTG	ATCTGGTGGG	AGGAGGGCAA	GATGACGGCC	480
AAGGTCGTAG	AGAGCGCCAA	GGCCATCCTG	GGCGGAAGCA	AGGTGCGCGT	GGACCAAAAG	540
TGCAAGTCAT	CGGCCAGAT	CGACCCAACT	CCCGTGATCG	TCACCTCCAA	CACCAACATG	600
TGCGCGGTCA	TCGACGGAAA	CTCGACCACC	TTCGAGCACC	AACAACCACT	CCAGGACCGG	660
ATGTTCAAGT	TCGAGCTCAC	CAAGCGCCTG	GAGCAGCACT	TTGGCAAGGT	CACCAAGCAG	720
GAAGTCAAAG	ACTTTTCCG	GTGGGCGTCA	GATCACGTGA	CCGAGGTGAC	TCACGAGTTT	780
TACGTCAGAA	AGGGTGGAGC	TAGAAAGAGG	CCCGCCCCCA	ATGACGCAGA	TATAAGTGAG	840
CCCAAGCGGG	CCTGTCCGTC	AGTTGCGCAG	CCATCGACGT	CAGACGCGGA	AGCTCCGGTG	900
GACTACGCGG	ACAGATTGGC	TAGAGGACAA	CCTCTCTGA			939

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1197 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ix) OTHER INFO: AAV4 Rep 52 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATGGAGCTGG	TCGGGTGGCT	GGTGGACCGC	GGGATCACGT	CAGAAAAGCA	ATGGATCCAG	60
GAGGACCAGG	CGTCCTACAT	CTCCTTCAAC	GCCGCCTCCA	ACTCGCGGTC	ACAAATCAAG	120
GCCGCGCTGG	ACAATGCCTC	CAAAATCATG	AGCCTGACAA	AGACGGCTCC	GGACTACCTG	180
GTGGGCCAGA	ACCCGCCGGA	GGACATTTCC	AGCAACCGCA	TCTACCGAAT	CCTCGAGATG	240

AACGGGTACG	ATCCGCAGTA	CGCGGCCTCC	GTCTTCCTGG	GCTGGGCGCA	AAAGAAGTTC	300
GGGAAGAGGA	ACACCATCTG	GCTCTTTGGG	CCGGCCACGA	CGGGTAAAAC	CAACATCGCG	360
GAAGCCATCG	CCCACGCCGT	GCCCTTCTAC	GGCTGCGTGA	ACTGGACCAA	TGAGAACTTT	420
CCGTTCAACG	ATTGCGTCGA	CAAGATGGTG	ATCTGGTGGG	AGGAGGGCAA	GATGACGGCC	480
AAGGTCGTAG	AGAGCGCCAA	GGCCATCCTG	GGCGGAAGCA	AGGTGCGCGT	GGACCAAAAG	540
TGCAAGTCAT	CGGCCCAGAT	CGACCCAACT	CCCCTGATCG	TCACCTCCAA	CACCAACATG	600
TGCGCGGTCA	TCGACGGAAA	CTCGACCACC	TTCGAGCACC	AACAACCACT	CCAGGACCGG	660
ATGTTCAAGT	TCGAGCTCAC	CAAGCGCCTG	GAGCAGCACT	TTGGCAAGGT	CACCAAGCAG	720
GAAGTCAAAG	ACTTTTTCCG	GTGGGCGTCA	GATCACGTGA	CCGAGGTGAC	TCACGAGTTT	780
TACGTCAGAA	AGGGTGGAGC	TAGAAAGAGG	CCCGCCCCCA	ATGACGCAGA	TATAAGTGAG	840
CCCAAGCGGG	CCTGTCCGTC	AGTTGCGCAG	CCATCGACGT	CAGACGCGGA	AGCTCCGGTG	900
GACTACGCGG	ACAGGTACCA	AAACAAATGT	TCTCGTCACG	TGGGTATGAA	TCTGATGCTT	960
TTTCCCTGCC	GGCAATGCGA	GAGAATGAAT	CAGAATGTGG	ACATTTGCTT	CACGCACGGG	1020
GTCATGGACT	GTGCCGAGTG	CTTCCCCGTG	TCAGAATCTC	AACCCGTGTC	TGTCGTCAGA	1080
AAGCGGACGT	ATCAGAAACT	GTGTCCGATT	CATCACATCA	TGGGGAGGGC	GCCCAGGTG	1140
GCCTGCTCGG	CCTGCGAACT	GGCCAATGTG	GACTTGGATG	ACTGTGACAT	GGAACAA	1197

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1611 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ix) OTHER INFO: AAV4 Rep 68 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ATGCCGGGGT	TCTACGAGAT	CGTGCTGAAG	GTGCCCAGCG	ACCTGGACGA	GCACCTGCCC	60
GGCATTCTTG	ACTCTTTTGT	GAGCTGGGTG	GCCGAGAAGG	AATGGGAGCT	GCCGCCGGAT	120
TCTGACATGG	ACTTGAATCT	GATTGAGCAG	GCACCCCTGA	CCGTGGCCGA	AAAGCTGCAA	180
CGCGAGTTCC	TGGTTCGAGT	GCGCCGCGTG	AGTAAGGCCC	CGGAGGCCCT	CTTCTTTGTC	240
CAGTTCGAGA	AGGGGGACAG	CTACTTCCAC	CTGCACATCC	TGGTGGAGAC	CGTGGGCGTC	300
AAATCCATGG	TGGTGGGCCG	CTACGTGAGC	CAGATTAAAG	AGAAGCTGGT	GACCCGCATC	360
TACCGCGGGG	TCGAGCCGCA	GCTTCCGAAC	TGGTTCGCGG	TGACCAAGAC	GCGTAATGGC	420
GCCGGAGGCG	GGAACAAGGT	GGTGGACGAC	TGCTACATCC	CCAACTACCT	GCTCCCCAAG	480
ACCCAGCCCG	AGCTCCAGTG	GGCGTGGACT	AACATGGACC	AGTATATAAG	CGCCTGTTTG	540
AATCTCGCGG	AGCGTAAACG	GCTGGTGGCG	CAGCATCTGA	CGCACGTGTC	GCAGACGCAG	600
GAGCAGAACA	AGGAAAACCA	GAACCCCAAT	TCTGACGCGC	CGGTTCATCAG	GTCAAAAACC	660
TCCGCCAGGT	ACATGGAGCT	GGTCGGGTGG	CTGGTGGACC	GCGGGATCAC	GTCAGAAAAG	720
CAATGGATCC	AGGAGGACCA	GGCGTCCCTAC	ATCTCCTTCA	ACGCCGCCTC	CAACTCGCGG	780
TCACAAATCA	AGGCCGCGCT	GGACAATGCC	TCCAAAATCA	TGAGCCTGAC	AAAGACGGCT	840
CCGGAATACC	TGGTGGGCCA	GAACCCGCCG	GAGGACATTT	CCAGCAACCG	CATCTACCGA	900
ATCCTCGAGA	TGAACGGGTA	CGATCCGCAG	TACGCGGCCT	CCGTCTTCCT	GGGCTGGGCG	960
CAAAAGAAGT	TCGGGAAGAG	GAACACCATC	TGGCTCTTTG	GGCCGGCCAC	GACGGGTAAA	1020
ACCAACATCG	CGGAAGCCAT	CGCCCACGCC	GTGCCCTTCT	ACGGCTGCGT	GAAGTGGACC	1080
AATGAGAACT	TTCCGTTCAA	CGATTGCGTC	GACAAGATGG	TGATCTGGTG	GGAGGAGGGC	1140
AAGATGACGG	CCAAGGTCGT	AGAGAGCGCC	AAGGCCATCC	TGGGCGGAAG	CAAGGTGCGC	1200
GTGGACCAAA	AGTGCAAGTC	ATCGGCCCAG	ATCGACCCAA	CTCCCGTGAT	CGTCACCTCC	1260
AACACCAACA	TGTGCGCGGT	CATCGACGGA	AACTCGACCA	CCTTCGAGCA	CCAACAACCA	1320
CTCCAGGACC	GGATGTTCAA	GTTCGAGCTC	ACCAAGCGCC	TGGAGCACGA	CTTTGGCAAG	1380
GTCACCAAGC	AGGAAGTCAA	AGACTTTTTC	CGGTGGGCGT	CAGATCACGT	GACCGAGGTG	1440
ACTCACGAGT	TTTACGTCAG	AAAGGGTGGG	GCTAGAAAGA	GGCCCGCCCC	CAATGACGCA	1500
GATATAAGTG	AGCCCAAGCG	GGCCTGTCCG	TCAGTTGCGC	AGCCATCGAC	GTCAGACGCG	1560
GAAGCTCCGG	TGGACTACGC	GGACAGATTG	GCTAGAGGAC	AACCTCTCTG	A	1611

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1872 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ix) OTHER INFO: AAV4 Rep 78 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATGCCGGGGT	TCTACGAGAT	CGTGCTGAAG	GTGCCCAGCG	ACCTGGACGA	GCACCTGCCC	60
GGCATTCTG	ACTCTTTTGT	GAGCTGGGTG	GCCGAGAAGG	AATGGGAGCT	GCCGCCGGAT	120
TCTGACATGG	ACTTGAATCT	GATTGAGCAG	GCACCCCTGA	CCGTGGCCGA	AAAGCTGCAA	180
CGCGAGTTCC	TGGTCGAGTG	GCGCCGCGTG	AGTAAGGCCC	CGGAGGCCCT	CTTCTTTGTC	240
CAGTTCGAGA	AGGGGGACAG	CTACTTCCAC	CTGCACATCC	TGGTGGAGAC	CGTGGGCGTC	300
AAATCCATGG	TGGTGGGCGG	CTACGTGAGC	CAGATTAAAG	AGAAGCTGGT	GACCCGCATC	360
TACCGCGGGG	TCGAGCCGCA	GCTTCCGAAC	TGGTTCGCGG	TGACCAAGAC	GCGTAATGGC	420
GCCGGAGGCG	GGAAACAAGGT	GGTGGACGAC	TGCTACATCC	CCAACTACCT	GCTCCCCAAG	480
ACCCAGCCCCG	AGCTCCAGTG	GGCGTGGACT	AACATGGACC	AGTATATAAG	CGCCTGTTTG	540
AATCTCGCGG	AGCGTAAACG	GCTGGTGGCG	CAGCATCTGA	CGCACGTGTC	GCAGACGCAG	600
GAGCAGAACA	AGGAAAACCA	GAACCCCAAT	TCTGACGCGC	CGGTTCATCAG	GTCAAAAACC	660
TCCGCCAGGT	ACATGGAGCT	GGTCGGGTGG	CTGGTGGACC	GCGGGATCAC	GTCAGAAAAG	720
CAATGGATCC	AGGAGGACCA	GGCGTCTTAC	ATCTCCTTCA	ACGCCGCCTC	CAACTCGCGG	780
TCACAAATCA	AGGCCGCGCT	GGACAATGCC	TCCAAAATCA	TGAGCCTGAC	AAAGACGGCT	840
CCGGACTACC	TGGTGGGCCA	GAACCCGCCG	GAGGACATTT	CCAGCAACCG	CATCTACCGA	900
ATCCTCGAGA	TGAACGGGTA	CGATCCGCAG	TACGCGGCCT	CCGTCTTCCT	GGGCTGGGCG	960
CAAAAGAAGT	TCGGGAAGAG	GAACACCATC	TGGCTCTTTG	GGCCGGCCAC	GACGGGTAAA	1020
ACCAACATCG	CGGAAGCCAT	CGCCCACGCC	GTGCCCTTCT	ACGGCTGCGT	GAAGTGGACC	1080
AATGAGAACT	TTCCGTTCAA	CGATTGCGTC	GACAAGATGG	TGATCTGGTG	GGAGGAGGGC	1140
AAGATGACGG	CCAAGTCTGT	AGAGAGCGCC	AAGGCCATCC	TGGGCGGAAG	CAAGGTGCGC	1200
GTGGACCAAA	AGTGCAAGTC	ATCGGCCCAG	ATCGACCCAA	CTCCCGTGAT	CGTCACCTCC	1260
AACACCAACA	TGTGCGCGGT	CATCGACGGA	AACTCGACCA	CCTTCGAGCA	CCAACAACCA	1320
CTCCAGGACC	GGATGTTCAA	GTTCGAGCTC	ACCAAGCGCC	TGGAGCACGA	CTTTGGCAAAG	1380
GTCACCAAGC	AGGAAGTCAA	AGACTTTTTC	CGGTGGGCGT	CAGATCACGT	GACCGAGGTG	1440
ACTCACGAGT	TTTACGTCAG	AAAGGTGGA	GCTAGAAAGA	GGCCCCGCCC	CAATGACGCA	1500
GATATAAGTG	AGCCCAAGCG	GGCCTGTCCG	TCAGTTGCGC	AGCCATCGAC	GTCAGACGCG	1560
GAAGCTCCGG	TGGACTACGC	GGACAGGTAC	CAAAACAAAT	GTTCTCGTCA	CGTGGGTATG	1620
AATCTGATGC	TTTTTGEETG	CCGGCAATGC	GAGAGAATGA	ATCAGAATGT	GGACATTGTC	1680
TTCACGCACG	GGGTCATGGA	CTGTGCCGAG	TGCTTCCCCG	TGTCAGAATC	TCAACCCGTG	1740
TCTGTCTGTC	GAAAGCGGAC	GTATCAGAAA	CTGTGTCCGA	TTCATCACAT	CATGGGGAGG	1800
GCGCCCGAGG	TGGCCTGCTC	GGCCTGCGAA	CTGGCCAATG	TGGACTTGGA	TGACTGTGAC	1860
ATGGAACAAT	AA					1872

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 598 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE:

(A) DESCRIPTION: protein

(ix) OTHER INFO: AAV4 capsid protein VP2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Thr	Ala	Pro	Gly	Lys	Lys	Arg	Pro	Leu	Ile	Glu	Ser	Pro	Gln	Gln	Pro
1				5					10					15	
Asp	Ser	Ser	Thr	Gly	Ile	Gly	Lys	Lys	Gly	Lys	Gln	Pro	Ala	Lys	Lys
			20				25						30		

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Lys	Leu	Val	Phe	Glu	Asp	Glu	Thr	Gly	Ala	Gly	Asp	Gly	Pro	Pro	Glu
	35						40				45				
Gly	Ser	Thr	Ser	Gly	Ala	Met	Ser	Asp	Asp	Ser	Glu	Met	Arg	Ala	Ala
	50					55					60				
Ala	Gly	Gly	Ala	Ala	Val	Glu	Gly	Gly	Gln	Gly	Ala	Asp	Gly	Val	Gly
65					70					75					80
Asn	Ala	Ser	Gly	Asp	Trp	His	Cys	Asp	Ser	Thr	Trp	Ser	Glu	Gly	His
			85						90					95	
Val	Thr	Thr	Thr	Ser	Thr	Arg	Thr	Trp	Val	Leu	Pro	Thr	Tyr	Asn	Asn
			100					105					110		
His	Leu	Tyr	Lys	Arg	Leu	Gly	Glu	Ser	Leu	Gln	Ser	Asn	Thr	Tyr	Asn
	115						120					125			
Gly	Phe	Ser	Thr	Pro	Trp	Gly	Tyr	Phe	Asp	Phe	Asn	Arg	Phe	His	Cys
	130					135					140				
His	Phe	Ser	Pro	Arg	Asp	Trp	Gln	Arg	Leu	Ile	Asn	Asn	Asn	Trp	Gly
145					150					155					160
Met	Arg	Pro	Lys	Ala	Met	Arg	Val	Lys	Ile	Phe	Asn	Ile	Gln	Val	Lys
			165					170						175	
Glu	Val	Thr	Thr	Ser	Asn	Gly	Glu	Thr	Thr	Val	Ala	Asn	Asn	Leu	Thr
		180						185					190		
Ser	Thr	Val	Gln	Ile	Phe	Ala	Asp	Ser	Ser	Tyr	Glu	Leu	Pro	Tyr	Val
	195						200					205			
Met	Asp	Ala	Gly	Gln	Glu	Gly	Ser	Leu	Pro	Pro	Phe	Pro	Asn	Asp	Val
	210					215					220				
Phe	Met	Val	Pro	Gln	Tyr	Gly	Tyr	Cys	Gly	Leu	Val	Thr	Gly	Asn	Thr
225					230					235					240
Ser	Gln	Gln	Gln	Thr	Asp	Arg	Asn	Ala	Phe	Tyr	Cys	Leu	Glu	Tyr	Phe
				245					250					255	
Pro	Ser	Gln	Met	Leu	Arg	Thr	Gly	Asn	Asn	Phe	Glu	Ile	Thr	Tyr	Ser
		260						265					270		
Phe	Glu	Lys	Val	Pro	Phe	His	Ser	Met	Tyr	Ala	His	Ser	Gln	Ser	Leu
	275						280					285			
Asp	Arg	Leu	Met	Asn	Pro	Leu	Ile	Asp	Gln	Tyr	Leu	Trp	Gly	Leu	Gln
	290					295					300				
Ser	Thr	Thr	Thr	Gly	Thr	Thr	Leu	Asn	Ala	Gly	Thr	Ala	Thr	Thr	Asn
305					310					315					320
Phe	Thr	Lys	Leu	Arg	Pro	Thr	Asn	Phe	Ser	Asn	Phe	Lys	Lys	Asn	Trp
			325						330					335	
Leu	Pro	Gly	Pro	Ser	Ile	Lys	Gln	Gln	Gly	Phe	Ser	Lys	Thr	Ala	Asn
		340						345					350		
Gln	Asn	Tyr	Lys	Ile	Pro	Ala	Thr	Gly	Ser	Asp	Ser	Leu	Ile	Lys	Tyr
	355						360					365			
Glu	Thr	His	Ser	Thr	Leu	Asp	Gly	Arg	Trp	Ser	Ala	Leu	Thr	Pro	Gly
	370					375					380				
Pro	Pro	Met	Ala	Thr	Ala	Gly	Pro	Ala	Asp	Ser	Lys	Phe	Ser	Asn	Ser
385					390					395					400
Gln	Leu	Ile	Phe	Ala	Gly	Pro	Lys	Gln	Asn	Gly	Asn	Thr	Ala	Thr	Val
			405						410					415	
Pro	Gly	Thr	Leu	Ile	Phe	Thr	Ser	Glu	Glu	Glu	Leu	Ala	Ala	Thr	Asn
		420						425					430		
Ala	Thr	Asp	Thr	Asp	Met	Trp	Gly	Asn	Leu	Pro	Gly	Gly	Asp	Gln	Ser
	435						440					445			
Asn	Ser	Asn	Leu	Pro	Thr	Val	Asp	Arg	Leu	Thr	Ala	Leu	Gly	Ala	Val
	450					455					460				
Pro	Gly	Met	Val	Trp	Gln	Asn	Arg	Asp	Ile	Tyr	Tyr	Gln	Gly	Pro	Ile
465					470					475					480
Trp	Ala	Lys	Ile	Pro	His	Thr	Asp	Gly	His	Phe	His	Pro	Ser	Pro	Leu
			485						490					495	
Ile	Gly	Gly	Phe	Gly	Leu	Lys	His	Pro	Pro	Pro	Gln	Ile	Phe	Ile	Lys
		500						505					510		
Asn	Thr	Pro	Val	Pro	Ala	Asn	Pro	Ala	Thr	Thr	Phe	Ser	Ser	Thr	Pro
	515						520					525			

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Val Asn Ser Phe Ile Thr Gln Tyr Ser Thr Gly Gln Val Ser Val Gln
 530 535 540
 Ile Asp Trp Glu Ile Gln Lys Glu Arg Ser Lys Arg Trp Asn Pro Glu
 545 550 555 560
 Val Gln Phe Thr Ser Asn Tyr Gly Gln Gln Asn Ser Leu Leu Trp Ala
 565 570 575
 Pro Asp Ala Ala Gly Lys Tyr Thr Glu Pro Arg Ala Ile Gly Thr Arg
 580 585 590
 Tyr Leu Thr His His Leu
 595

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1800 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ix) OTHER INFO: AAV4 capsid protein VP2 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ACGGCTCCTG	GAAAGAAGAG	ACCGTTGATT	GAATCCCCCC	AGCAGCCCGA	CTCCTCCACG	60
GGTATCGGCA	AAAAAGGCAA	GCAGCCGGCT	AAAAAGAAGC	TCGTTTTCGA	AGACGAAACT	120
GGAGCAGGCG	ACGGACCCCC	TGAGGGATCA	ACTTCCGGAG	CCATGTCTGA	TGACAGTGAG	180
ATGCGTGCG	CAGCTGGCGG	AGCTGCAGTC	GAGGGSGGAC	AAGGTGCCGA	TGGAGTGGGT	240
AATGCCTCGG	GTGATTGGCA	TTGCGATTCC	ACCTGGTCTG	AGGGCCACGT	CACGACCACC	300
AGCACCAGAA	CCTGGGTCTT	GCCACCTAC	AACAACCACC	TNTACAAGCG	ACTCGGAGAG	360
AGCCTGCAGT	CCAACACCTA	CAACGGATTG	TCCACCCCCT	GGGGATACTT	TGACTTCAAC	420
CGCTTCCACT	GCCACTTCTC	ACCACGTGAC	TGGCAGCGAC	TCATCAACAA	CAACTGGGGC	480
ATGCGACCCA	AAGCCATGCG	GGTCAAAATC	TTCAACATCC	AGGTCAAGGA	GGTCACGACG	540
TCGAACGGCG	AGACAACGGT	GGCTAATAAC	CTTACCAGCA	CGGTTTCAGT	CTTTGCCGGAC	600
TCGTCGTACG	AACTGEEGTA	EGTGATGGAT	GCGGGTCAAG	AGGGCAGCCT	GCCTCCTTTT	660
CCCAACGACG	TCTTTATGGT	GCCCCAGTAC	GGCTACTGTG	GACTGGTGAC	CGGCAACACT	720
TCGCAGCAAC	AGACTGACAG	AAATGCCTTC	TACTGCCTGG	AGTACTTTCC	TTTCGAGATG	780
CTGCGGACTG	GCAACAACCT	TGAAATTACG	TACAGTTTTG	AGAAGGTGCC	TTTCCACTCG	840
ATGTACGCGC	ACAGCCAGAG	CCTGGACCGG	CTGATGAACC	CTCTCATCGA	CCAGTACCTG	900
TGGGGACTGC	AATCGACCAC	CACCGGAACC	ACCCTGAATG	CCGGGACTGC	CACCACCAAC	960
TTTACCAAGC	TGCGGCCTAC	CAACTTTTCC	AACTTTAAAA	AGAACTGGCT	GCCCCGGGCCT	1020
TCAATCAAGC	AGCAGGGCTT	CTCAAAGACT	GCCAAATCAA	ACTACAAGAT	CCCTGCCACC	1080
GGGTCAGACA	GTCTCATCAA	ATACGAGACG	CACAGCACTC	TGGACGGAAG	ATGGAGTGCC	1140
CTGACCCCGG	GACCTCCAAT	GGCCACGGCT	GGACCTGCGG	ACAGCAAGTT	CAGCAACAGC	1200
CAGCTCATCT	TTGCGGGGCC	TAAACAGAAC	GGCAACACGG	CCACCGTACC	CGGGACTCTG	1260
ATCTTCACCT	CTGAGGAGGA	GCTGGCAGCC	ACCAACGCCA	CCGATACGGA	CATGTGGGGC	1320
AACCTACCTG	GCGGTGACCA	GAGCAACAGC	AACCTGCCGA	CCGTGGACAG	ACTGACAGCC	1380
TTGGGAGCCG	TGCCTGGAAT	GGTCTGGCAA	AACAGAGACA	TTTACTACCA	GGGTCCCATT	1440
TGGGCCAAGA	TTCTTCATAC	CGATGGACAC	TTTACCCCCT	CACCGCTGAT	TGGTGGGTTT	1500
GGGCTGAAAC	ACCGCCTCC	TCAAATTTT	ATCAAGAACA	CCCCGGTACC	TGCGAATCCT	1560
GCAACGACCT	TCAGCTCTAC	TCCGGTAAAC	TCCTTCATTA	CTCAGTACAG	CAGTGCCACG	1620
GTGTGGGTGC	AGATTGACTG	GGAGATCCAG	AAGGAGCGGT	CCAAACGCTG	GAACCCCGAG	1680
GTCCAGTTTA	CCTCCAATA	CGGACAGCAA	AACCTCTCTG	TGTGGGCTCC	CGATGCGGCT	1740
GGGAAATACA	CTGAGCCTAG	GGCTATCGGT	ACCCGCTACC	TCACCCACCA	CCTGTAATAA	1800

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 544 amino acids
 (B) TYPE: amino acid

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(C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE:
 (A) DESCRIPTION: protein

(ix) OTHER INFO: AAV4 capsid protein VP3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met	Ser	Asp	Asp	Ser	Glu	Met	Arg	Ala	Ala	Ala	Gly	Gly	Ala	Ala	Val	1	5	10	15
Glu	Gly	Gly	Gln	Gly	Ala	Asp	Gly	Val	Gly	Asn	Ala	Ser	Gly	Asp	Trp	20	25	30	
His	Cys	Asp	Ser	Thr	Trp	Ser	Glu	Gly	His	Val	Thr	Thr	Thr	Ser	Thr	35	40	45	
Arg	Thr	Trp	Val	Leu	Pro	Thr	Tyr	Asn	Asn	His	Leu	Tyr	Lys	Arg	Leu	50	55	60	
Gly	Glu	Ser	Leu	Gln	Ser	Asn	Thr	Tyr	Asn	Gly	Phe	Ser	Thr	Pro	Trp	65	70	75	80
Gly	Tyr	Phe	Asp	Phe	Asn	Arg	Phe	His	Cys	His	Phe	Ser	Pro	Arg	Asp	85	90	95	
Trp	Gln	Arg	Leu	Ile	Asn	Asn	Asn	Trp	Gly	Met	Arg	Pro	Lys	Ala	Met	100	105	110	
Arg	Val	Lys	Ile	Phe	Asn	Ile	Gln	Val	Lys	Glu	Val	Thr	Thr	Ser	Asn	115	120	125	
Gly	Glu	Thr	Thr	Val	Ala	Asn	Asn	Leu	Thr	Ser	Thr	Val	Gln	Ile	Phe	130	135	140	
Ala	Asp	Ser	Ser	Tyr	Glu	Leu	Pro	Tyr	Val	Met	Asp	Ala	Gly	Gln	Glu	145	150	155	160
Gly	Ser	Leu	Pro	Pro	Phe	Pro	Asn	Asp	Val	Phe	Met	Val	Pro	Gln	Tyr	165	170	175	
Gly	Tyr	Cys	Gly	Leu	Val	Thr	Gly	Asn	Thr	Ser	Gln	Gln	Gln	Thr	Asp	180	185	190	
Arg	Asn	Ala	Phe	Tyr	Cys	Leu	Glu	Tyr	Phe	Pro	Ser	Gln	Met	Leu	Arg	195	200	205	
Thr	Gly	Asn	Asn	Phe	Glu	Ile	Thr	Tyr	Ser	Phe	Glu	Lys	Val	Pro	Phe	210	215	220	
His	Ser	Met	Tyr	Ala	His	Ser	Gln	Ser	Leu	Asp	Arg	Leu	Met	Asn	Pro	225	230	235	240
Leu	Ile	Asp	Gln	Tyr	Leu	Trp	Gly	Leu	Gln	Ser	Thr	Thr	Thr	Gly	Thr	245	250	255	
Thr	Leu	Asn	Ala	Gly	Thr	Ala	Thr	Thr	Asn	Phe	Thr	Lys	Leu	Arg	Pro	260	265	270	
Thr	Asn	Phe	Ser	Asn	Phe	Lys	Lys	Asn	Trp	Leu	Pro	Gly	Pro	Ser	Ile	275	280	285	
Lys	Gln	Gln	Gly	Phe	Ser	Lys	Thr	Ala	Asn	Gln	Asn	Tyr	Lys	Ile	Pro	290	295	300	
Ala	Thr	Gly	Ser	Asp	Ser	Leu	Ile	Lys	Tyr	Glu	Thr	His	Ser	Thr	Leu	305	310	315	320
Asp	Gly	Arg	Trp	Ser	Ala	Leu	Thr	Pro	Gly	Pro	Pro	Met	Ala	Thr	Ala	325	330	335	
Gly	Pro	Ala	Asp	Ser	Lys	Phe	Ser	Asn	Ser	Gln	Leu	Ile	Phe	Ala	Gly	340	345	350	
Pro	Lys	Gln	Asn	Gly	Asn	Thr	Ala	Thr	Val	Pro	Gly	Thr	Leu	Ile	Phe	355	360	365	
Thr	Ser	Glu	Glu	Glu	Leu	Ala	Ala	Thr	Asn	Ala	Thr	Asp	Thr	Asp	Met	370	375	380	
Trp	Gly	Asn	Leu	Pro	Gly	Gly	Asp	Gln	Ser	Asn	Ser	Asn	Leu	Pro	Thr	385	390	395	400
Val	Asp	Arg	Leu	Thr	Ala	Leu	Gly	Ala	Val	Pro	Gly	Met	Val	Trp	Gln	405	410	415	

Asn Arg Asp Ile Tyr Tyr Gln Gly Pro Ile Trp Ala Lys Ile Pro His
 420 425 430
 Thr Asp Gly His Phe His Pro Ser Pro Leu Ile Gly Gly Phe Gly Leu
 435 440 445
 Lys His Pro Pro Pro Gln Ile Phe Ile Lys Asn Thr Pro Val Pro Ala
 450 455 460
 Asn Pro Ala Thr Thr Phe Ser Ser Thr Pro Val Asn Ser Phe Ile Thr
 465 470 475 480
 Gln Tyr Ser Thr Gly Gln Val Ser Val Gln Ile Asp Trp Glu Ile Gln
 485 490 495
 Lys Glu Arg Ser Lys Arg Trp Asn Pro Glu Val Gln Phe Thr Ser Asn
 500 505 510
 Tyr Gly Gln Gln Asn Ser Leu Leu Trp Ala Pro Asp Ala Ala Gly Lys
 515 520 525
 Tyr Thr Glu Pro Arg Ala Ile Gly Thr Arg Tyr Leu Thr His His Leu
 530 535 540

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1617 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ix) OTHER INFO: AAV4 capsid protein VP3 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ATGCGTGCGAG	CAGCTGGCGG	AGCTGCAGTC	GAGGSGGGAC	AAGGTGCCGA	TGGAGTGGGT	60
AATGCCTCGG	GTGATTGGCA	TTGCGATTCC	ACCTGGTCTG	AGGGCCACGT	CACGACCACC	120
AGCACCAGAA	CCTGGGTCTT	GGCCACCTAC	AACAACCACC	TNTACAAGCG	ACTCGGAGAG	180
AGCCTGCAGT	CCAACACCTA	CAACGGATTG	TCCACCCCTT	GGGGATACTT	TGACTTCAAC	240
CGCTTCCACT	GCCACTTCTC	ACCACGTGAC	TGGCAGCGAC	TCATCAACAA	CAACTGGGGC	300
ATGCGACCCA	AAGGEATGCG	GGTCAAAATC	TTCAACATCC	AGGTCAAGGA	GGTCACGACG	360
TCGAACGGCG	AGACAACGGT	GGCTAATAAC	CTTACCAGCA	CGGTTTCAGT	CTTTGCGGAC	420
TCGTGCTACG	AACTGCCGTA	CGTGATGGAT	GCGGGTCAAG	AGGGCAGCCT	GCCTCCTTTT	480
CCCAACGACG	TCTTTATGGT	GGCCAGTAC	GGCTACTGTG	GACTIONGTG	CGGCAACACT	540
TCGCAGCAAC	AGACTGACAG	AAATGCCTTC	TACTGCCTGG	AGTACTTTCC	TTTCGAGATG	600
CTGCGGACTG	GCAACAACCT	TGAAATTACG	TACAGTTTTG	AGAAGGTGCC	TTTCCACTCG	660
ATGTACGCGC	ACAGCCAGAG	CCTGGACCGG	CTGATGAACC	CTCTCATCGA	CCAGTACCTG	720
TGGGGACTGC	AATCGACCAC	CACCGGAACC	ACCCTGAATG	CCGGGACTGC	CACCACCAAC	780
TTTACCAAGC	TGCGGCCTAC	CAACTTTTCC	AACCTTAAAA	AGAAGTGGCT	GCCCGGGCCT	840
TCAATCAAGC	AGCAGGGCTT	CTCAAAGACT	GCCAATCAAA	ACTACAAGAT	CCCTGCCACC	900
GGGTGAGACA	GTCTCATCAA	ATACGAGACG	CACAGCACTC	TGGACGGAAG	ATGGAGTGCC	960
CTGACCCCGG	GACCTCCAAT	GGCCACGGCT	GGACCTGCGG	ACAGCAAGTT	CAGCAACAGC	1020
CAGCTCATCT	TTGCGGGGCC	TAAACAGAAC	GGCAACACGG	CCACCGTACC	CGGGACTCTG	1080
ATCTTCACCT	CTGAGGAGGA	GCTGGCAGCC	ACCAACGCCA	CCGATACGGA	CATGTGGGGC	1140
AACCTACCTG	GCGGTGACCA	GAGCAACAGC	AACCTGCCGA	CCGTGGACAG	ACTGACAGCC	1200
TTGGGAGCCG	TGCCTGGAAT	GGTCTGGCAA	AACAGAGACA	TTTACTACCA	GGGTCCCATT	1260
TGGGCCAAGA	TTCTTCATAC	CGATGGACAC	TTTCACCCCT	CACCGCTGAT	TGGTGGGTTT	1320
GGGCTGAAAC	ACCCGCCTCC	TCAAATTTTT	ATCAAGAACA	CCCCGGTACC	TGCGAATCCT	1380
GCAACGACCT	TCAGCTCTAC	TCCGGTAAAC	TCCTTCATTA	CTCAGTACAG	CACCTGGCCAG	1440
GTGTCGGTGC	AGATTGACTG	GGAGATCCAG	AAGGAGCGGT	CCAAACGCTG	GAACCCCGAG	1500
GTCCAGTTTA	CCTCCAATA	CGGACAGCAA	AACCTCTCTG	TGTGGGCTCC	CGATGCGGCT	1560
GGGAAATACA	CTGAGCCTAG	GGCTATCGGT	ACCCGCTACC	TCACCCACCA	CCTGTAA	1617

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 129 base pairs

69

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) OTHER INFO: AAV4 ITR "flop" orientation

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TTGGCCACTC CCTCTATGCG CGCTCGCTCA CTCACTCGGC CCTGCGGCCA GAGGCCGGCA	60
GTCTGGAGAC CTTTGGTGTC CAGGGCAGGG CCGAGTGAGT GAGCGAGCGC GCATAGAGGG	120
AGTGGCCAA	129

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TCTAGTCTAG ACTTGGCCAC TCCCTCTCTG CGCGC	35
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(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AGGCCTTAAG AGCAGTCGTC CACCACCTTG TTCC	34
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What is claimed is:

1. A nucleic acid vector comprising a pair of adeno-associated virus 4 (AAV4) inverted terminal repeats and a promoter between the inverted terminal repeats.
 2. The vector of claim 1, wherein the AAV4 inverted terminal repeats comprise the nucleotide sequence set forth in SEQ ID NO: 6.
 3. The vector of claim 1, wherein the AAV4 inverted terminal repeats comprise the nucleotide sequence set forth in SEQ ID NO: 20.
 4. The vector of claim 1, wherein the promoter is an AAV promoter p5.
 5. The vector of claim 1, wherein the p5 promoter is AAV4 p5 promoter.
 6. The vector of claim 1, further comprising an exogenous nucleic acid functionally linked to the promoter.
-
7. The vector of claim 1 encapsidated in an adeno-associated virus particle.
 8. The particle of claim 7, wherein the particle is an AAV4 particle.
 9. The particle of claim 7, wherein the particle is an AAV1 particle, an AAV2 particle, an AAV3 particle or an AAV5 particle.
 10. An AAV4 particle containing a vector comprising a pair of AAV2 inverted terminal repeats.
 11. The particle of claim 10, wherein the vector further comprises an exogenous nucleic acid inserted between the inverted terminal repeats.

12. An isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:1.
13. An isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO:1.
14. An isolated nucleic acid that selectively hybridizes with the nucleic acid of claim 13.
15. An isolated nucleic acid encoding an adeno-associated virus 4 Rep protein.
16. The nucleic acid of claim 15, wherein the adeno-associated virus 4 Rep protein has the amino acid sequence set forth in SEQ ID NO:2.
17. The nucleic acid of claim 15, wherein the adeno-associated virus 4 Rep protein has the amino acid sequence set forth in SEQ ID NO:8.
18. The nucleic acid of claim 15, wherein the adeno-associated virus 4 Rep protein has the amino acid sequence set forth in SEQ ID NO:9.
19. The nucleic acid of claim 15, wherein the adeno-associated virus 4 Rep protein has the amino acid sequence set forth in SEQ ID NO:10.
20. The nucleic acid of claim 15, wherein the adeno-associated virus 4 Rep protein has the amino acid sequence set forth in SEQ ID NO:11.
21. The nucleic acid of claim 15, wherein the nucleic acid comprises the nucleotide sequence set forth in SEQ ID NO:3.
22. The nucleic acid of claim 15, wherein the nucleic acid consists essentially of the nucleotide sequence set forth in SEQ ID NO:3.
23. An isolated nucleic acid that selectively hybridizes with the nucleic acid of claim 22.

24. The nucleic acid of claim 15, wherein the nucleic acid comprises the nucleotide sequence set forth in SEQ ID NO:12.
25. The nucleic acid of claim 15, wherein the nucleic acid comprises the nucleotide sequence set forth in SEQ ID NO:13.
26. The nucleic acid of claim 15, wherein the nucleic acid comprises the nucleotide sequence set forth in SEQ ID NO:14.
27. The nucleic acid of claim 15, wherein the nucleic acid comprises the nucleotide sequence set forth in SEQ ID NO:15.
28. An isolated AAV4 Rep protein having the amino acid sequence set forth in SEQ ID NO:2, or a unique fragment thereof.
29. An isolated AAV4 Rep protein having the amino acid sequence set forth in SEQ ID NO:8, or a unique fragment thereof.

30. An isolated AAV4 Rep protein having the amino acid sequence set forth in SEQ ID NO:9, or a unique fragment thereof.
31. An isolated AAV4 Rep protein having the amino acid sequence set forth in SEQ ID NO:10, or a unique fragment thereof.
32. An isolated AAV4 Rep protein having the amino acid sequence set forth in SEQ ID NO:11, or a unique fragment thereof.
33. An isolated antibody that specifically binds the protein of claim 28.

34. An isolated AAV4 capsid protein having the amino acid sequence set forth in SEQ ID NO:4.
35. An isolated antibody that specifically binds the protein of claim 34.
36. An isolated AAV4 capsid protein having the amino acid sequence set forth in SEQ ID NO:16.
37. An isolated antibody that specifically binds the protein of claim 36.
38. An isolated AAV4 capsid protein having the amino acid sequence set forth in SEQ ID NO:18.
39. An isolated antibody that specifically binds the protein of claim 38.
40. An isolated nucleic acid encoding adeno-associated virus 4 capsid protein.
41. An isolated nucleic acid encoding the protein of claim 34.
42. The nucleic acid of claim 41, wherein the nucleic acid comprises the nucleic acid sequence set forth in SEQ ID NO:5.
43. The nucleic acid of claim 41, wherein the nucleic acid consists essentially of the nucleic acid sequence set forth in SEQ ID NO:5.
44. An isolated nucleic acid that selectively hybridizes with the nucleic acid of claim 39.
45. An AAV4 particle comprising a capsid protein consisting essentially of the amino acid sequence set forth in SEQ ID NO:4.
46. An isolated nucleic acid comprising an AAV4 p5 promoter.

47. A method of screening a cell for infectivity by AAV4 comprising contacting the cell with AAV4 and detecting the presence of AAV4 in the cells.
48. The method of claim 47, wherein the presence of AAV4 is detected in the cells by nucleic acid hybridization.
49. A method of determining the suitability of an AAV4 vector for administration to a subject comprising administering to an antibody-containing sample from the subject an antigenic fragment of the protein of claim 37 and detecting an antibody-antigen reaction in the sample, the presence of a reaction indicating the AAV4 vector to be unsuitable for use in the subject.
50. A method of determining the suitability of an AAV4 vector for administration to a subject comprising administering to an antibody-containing sample from the subject an antigenic fragment of the protein of claim 15 and detecting an antibody-antigen reaction in the sample, the presence of a reaction indicating the AAV4 vector to be unsuitable-for-use-in-the-subject.
51. A method of determining the presence in a subject of an AAV4-specific antibody comprising administering to an antibody-containing sample from the subject an antigenic fragment of the protein of claim 37 and detecting an antibody-antigen reaction in the sample, the presence of a reaction indicating the presence of an AAV4-specific antibody in the subject.
52. A method of delivering a nucleic acid to a cell comprising administering to the cell an AAV4 particle containing a vector comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, thereby delivering the nucleic acid to the cell.
53. The method of claim 52, wherein the AAV inverted terminal repeats are AAV4 inverted terminal repeats.

54. The method of claim 52, wherein the AAV inverted terminal repeats are AAV2 inverted terminal repeats.
55. A method of delivering a nucleic acid to a subject comprising administering to a cell from the subject an AAV4 particle comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, and returning the cell to the subject, thereby delivering the nucleic acid to the subject.
56. A method of delivering a nucleic acid to a cell in a subject comprising administering to the subject an AAV4 particle comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, thereby delivering the nucleic acid to a cell in the subject.
57. A method of delivering a nucleic acid to a cell in a subject having antibodies to AAV2 comprising administering to the subject an AAV4 particle comprising the nucleic acid, thereby delivering the nucleic acid to a cell in the subject.

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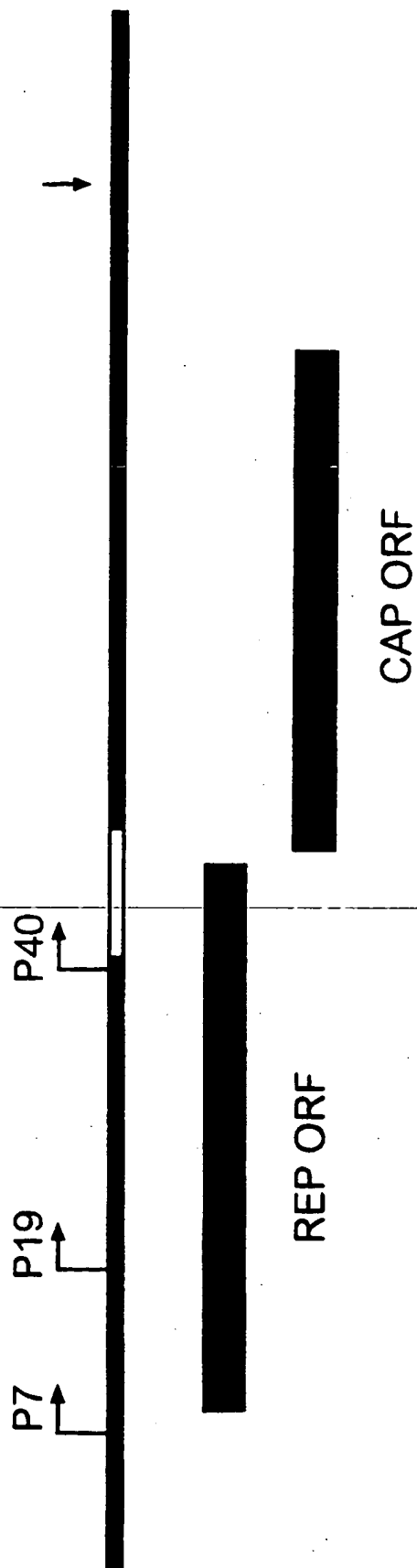


FIG 1

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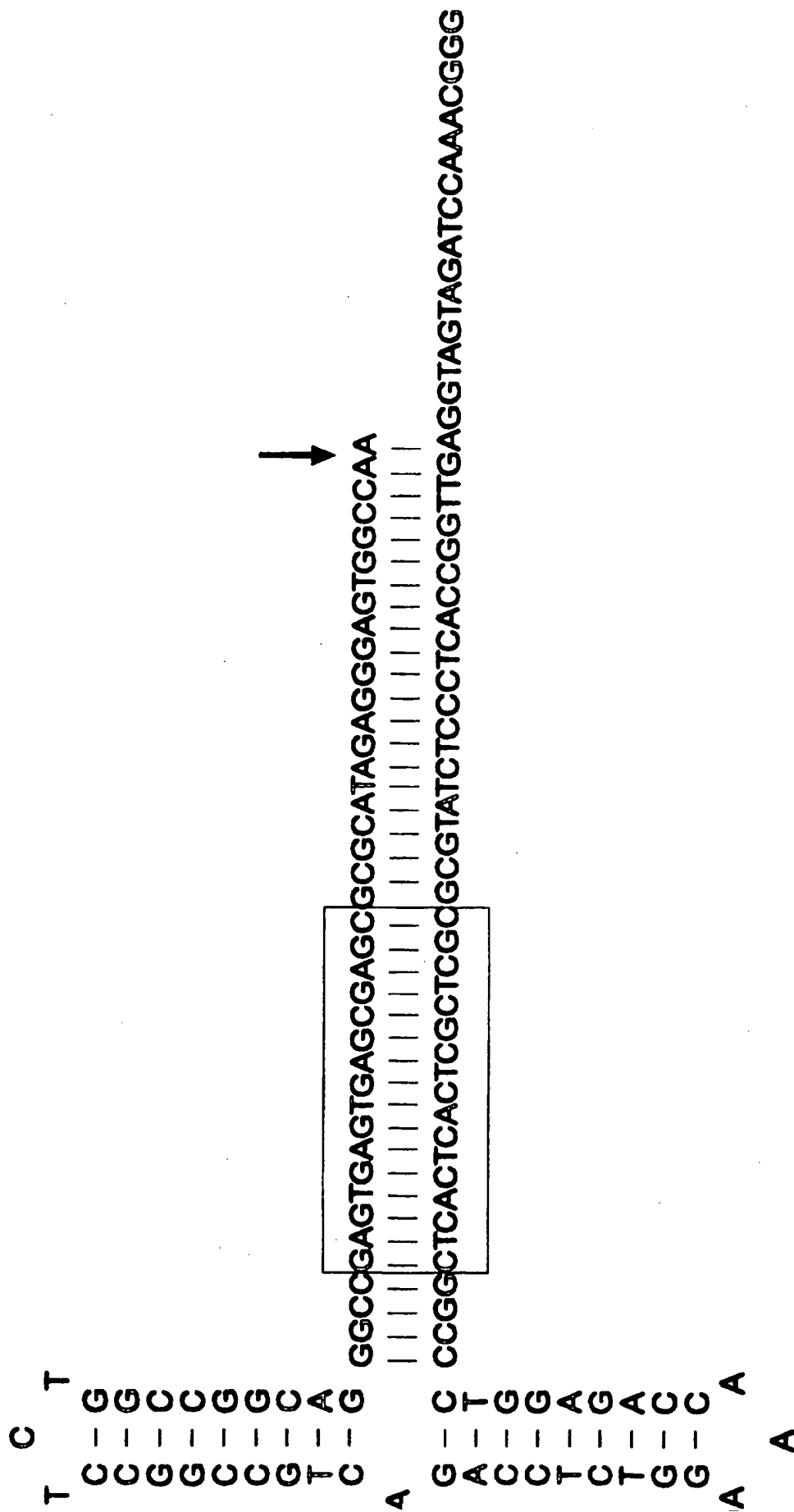
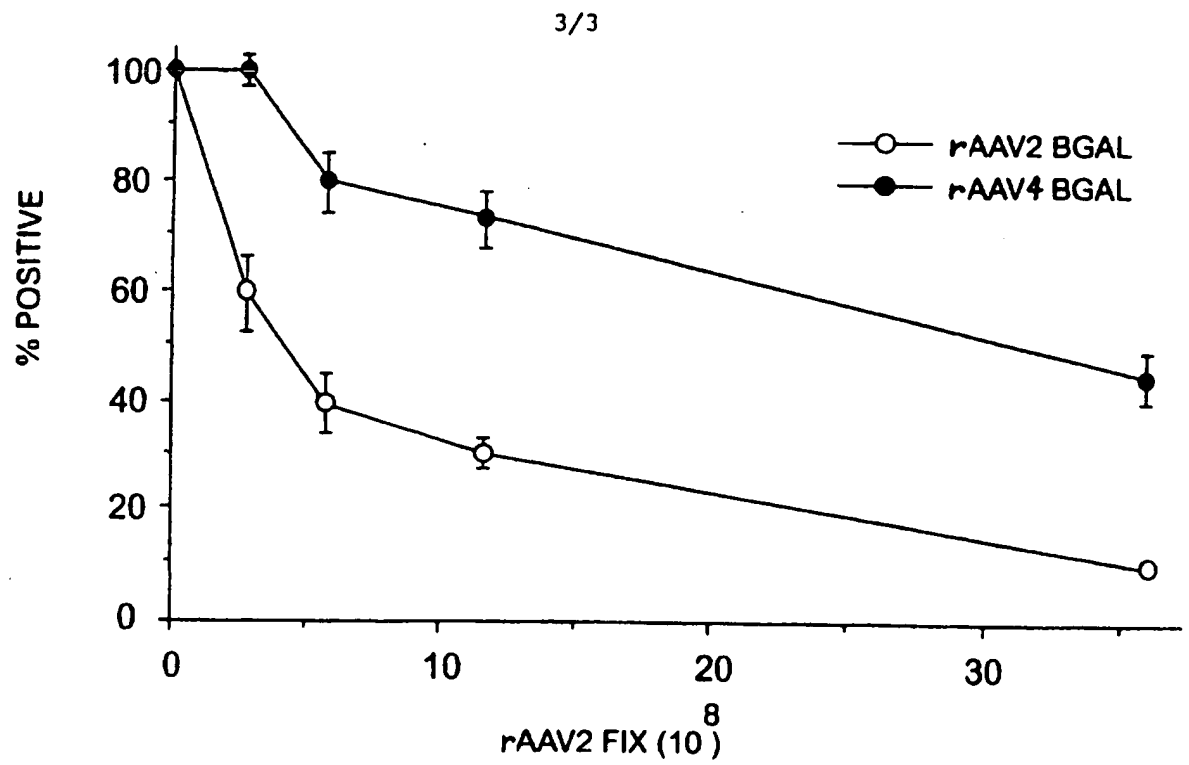
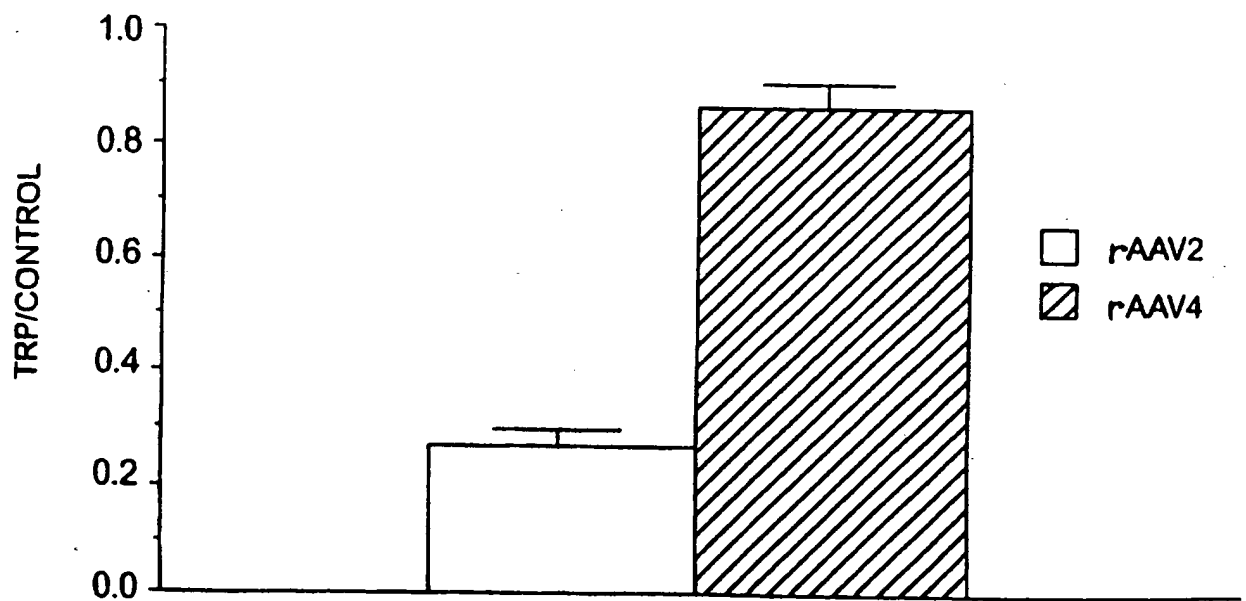


FIG.2

**FIG.3****FIG.4**

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶: C12N 15/86, C07K 14/015, G01N 33/53, A61K 48/00	A3	(11) International Publication Number: WO 98/11244 (43) International Publication Date: 19 March 1998 (19.03.98)
(21) International Application Number: PCT/US97/16266 (22) International Filing Date: 11 September 1997 (11.09.97) (30) Priority Data: 60/025,934 11 September 1996 (11.09.96) US (71) Applicant (for all designated States except US): THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; National Institutes of Health, Office of Technology Transfer, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): CHIORINI, John, A. [US/US]; 2604 Loma Street, Silver Spring, MD 20902 (US). KOTIN, Robert, M. [US/US]; 707 Gormley, Rockville, MD 20850 (US). SAFER, Brian [US/US]; 1610 Tilton Drive, Silver Springs, MD 20902 (US). (74) Agents: SELBY, Elizabeth et al.; Needle & Rosenberg, Suite 1200, 127 Peachtree Street, N.E., Atlanta, GA 30303 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 22 May 1998 (22.05.98)

(54) Title: AAV4 VECTOR AND USES THEREOF



(57) Abstract

The present invention provides an adeno-associated virus 4 (AAV4) virus and vectors and particles derived therefrom. In addition, the present invention provides methods of delivering a nucleic acid to a cell using the AAV4 vectors and particles.

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INTERNATIONAL SEARCH REPORT

Application No

PCT/US 97/16266

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/86 C07K14/015 G01N33/53 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 18727 A (AVIGEN INC) 20 June 1996 see page 9, paragraph 2 - paragraph 4 see page 10, paragraph 2 - paragraph 3 see page 11, line 13 - line 27 see page 32, line 5 - line 26; figure 7B; examples 1,2	1,10,15, 33,47, 48,52-54
Y	SHIN-ICHI MURAMATSU ET AL: "NUCLEOTIDE SEQUENCING AND GENERATION OF AN INFECTIOUS CLONE OF ADENO-ASSOCIATED VIRUS 3" VIROLOGY, -vol. 221, no. 1, 1 July 1996, pages 208-217, XP000608965 see the whole document	1,10,15, 33,40, 47,48, 52-54

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

12 March 1998

Date of mailing of the international search report

27. 03. 98

Name and mailing address of the ISA

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Internati Application No
PCT/US 97/16266

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/16266

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
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Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 55-57 and 52-54, as far as they concern an in vivo method , are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Application No

PCT/US 97/16266

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9618727 A	20-06-96	EP 0793713 A	10-09-97
WO 9600587 A	11-01-96	AU 2913895 A	25-01-96
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		EP 0766569 A	09-04-97

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